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TOXIC HAZARDS RESEARCH UNIT ANNUAL TECHNICAL REPORT: 1983

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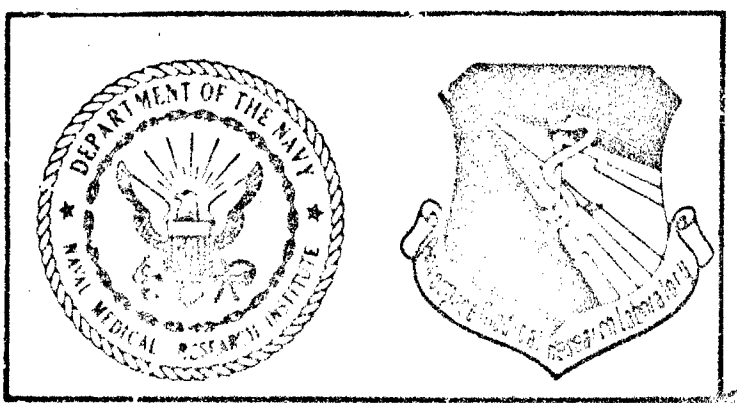

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TECHNICAL REVIEW AND APPROVAL

AFAMRL-TR-83-64

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

Michael D MacNaughton

MICHAEL G. MACNAUGHTON, LT COL, USAF, BSC
Deputy Director
Toxic Hazards Division
Air Force Aerospace Medical Research Laboratory

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			Shale JP-5	DFM	Hydraulic Fluids
			JP-7	JP-TS	JP-10

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The research program of the Toxic Hazards Research Unit (THRU) for the period of June 1982 through May 1983 is reviewed in this report. Chronic toxicity and oncogenic studies were carried out with hydrazine, Otto Fuel II, JP-7, JP-8, and JP-TS. A series of acute toxicity studies was conducted on a variety of chemicals and chemical agents used by the Army, Air Force, and Navy. Neurotoxicity and subchronic inhalation studies were conducted on several hydraulic fluids.

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18. SUBJECT TERMS

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Fuels
Inhalation
Toxicity
Acute
Chronic
Subchronic
Irritation
Skin
Percutaneous
Oral
Inhalation
Sensitization
Dermal
Shale Oil Fuels
Petroleum Fuels
Otto Fuel II
Neurotoxicity
Intraperitoneal
Antimony Thioantimonate
O-ethyl-O'-(2-diisopropylaminoethyl)methylphosphonite

PREFACE

This is the twentieth annual report of the Toxic Hazards Research Unit (THRU) and concerns work performed by the Department of Community and Environmental Medicine of the University of California, Irvine on behalf of the Air Force under Contract Number F33615-80-C-0512. This document constitutes the third report under the current contract and describes the accomplishments of the THRU from June 1982 through June 1983.

The current contract for operation of the Laboratory was initiated in 1980 under Project 6302, "Occupational and Environmental Toxic Hazards in Air Force Operations", Task 01, "Toxicology of Aerospace Chemicals and Materials", Work Unit Number 63020115. M. K. Pinkerton served as the technical contract monitor for the Air Force Aerospace Medical Research Laboratory.

This is a co-sponsored U. S. Air Force/U. S. Navy research effort. That portion of the work effort sponsored by the U. S. Navy was under the direction of LCDR L. Loring Pitts and Captain David E. Uddin, MSC, USN, and identified as Navy Task Area Number MF58524001 "Chemical Hazards/Exposure Limits".

J. D. MacEwen, Ph.D., served as Laboratory Director for the THRU of the University of California, Irvine and as co-principal investigator with T. T. Crocker, M.D., Professor and Chairman, Department of Community and Environmental Medicine. Acknowledgement is made to C. L. Gaworski, C. C. Haun, J. R. Horton, C. E. Johnson, E. R. Kinkead, P. E. Newton, Ph.D., A. K. Roychowdhury, Ph.D., and J. L. Monroe for their significant contributions and assistance in the preparation of this report. Partial support for this program was provided by the U. S. Naval Medical Research Institute, Toxicology Detachment, Wright-Patterson Air Force Base, Ohio, and the United States Army.

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SECTION I
INTRODUCTION

The research activity of the Toxic Hazards Research Unit (THRU) is a continuing program independent of contract years, with several studies in progress at the beginning and end of each report period. Experiments that were initiated and completed during the past year and were of sufficient magnitude to merit separate technical reports may only be summarized in this document. Unpublished letter reports are given in detail herein. This year's inhalation research program was conducted on a variety of fuels used for powering rockets, ships, torpedoes, aircraft, and on a variety of chemical mixtures used as hydraulic fluids. The results or current status of these studies will be discussed in the body of this report. Acute oral and dermal toxicity studies on a variety of materials were also conducted. Delayed neurotoxicity studies were also performed on several compounds.

This document constitutes the 20th annual report of the Toxic Hazards Research Unit, a research team which operates a dedicated inhalation toxicology laboratory to investigate potentially hazardous chemicals and materials of interest to the U. S. Air Force, U. S. Navy, and other governmental agencies. The THRU research team is an interdisciplinary group of University of California, Irvine, toxicologists, chemists, statisticians and engineers. Support services in pathology, veterinary medicine and medical technology are provided to the contract by the Air Force.

The research facilities used by the THRU consist of animal exposure chambers and supporting laboratories which have previously been described by MacEwen (1965), Fairchild (1967), and Thomas (1968).

During the first six years of operation, the primary research efforts of the THRU were directed to obtaining information on health hazards of spacecraft flight, and the biological data obtained have been used as criteria for setting continuous exposure limits and for engineering design factors. The primary research efforts have in recent years focused more on problems of aircraft environments, chronic occupational health problems, and the potential oncogenicity of chemicals used in military and civilian activities. To this end, the current research programs serve the mutual interests of the U. S. Air Force, U. S. Navy, U. S. Army and other governmental agencies.

ANNUAL CONFERENCE

As part of its contractual responsibilities, UCI/THRU presents an annual technical conference to disseminate new toxicologic information to the U. S. Air Force and other governmental and industrial scientists. This year's conference was chaired by James L. Whittenberger, M.D., Director of the Southern Occupational Health Center, University of California, Irvine. Twenty-seven technical platform papers were presented covering a wide variety of occupational and environmental toxicology problems. The open forum discussions following each session resulted in significant contributions of additional technical information and scientific exchange. The conference, held 16 November through 18 November, 1982, drew 178 participants including speakers.

The welcoming remarks were presented by Major General John W. Ord, USAF, MC, Commander, Aerospace Medical Division.

The conference program was submitted to the American Board of Industrial Hygiene and to the University of California, Irvine Continuing Education organization for evaluation. The ABIH awarded 2 1/2 points for recertification and 18 C.E.U.'s were awarded to attending physicians.

The papers presented at the conference were prepared for publication as the Proceedings of the 13th Conference on Environmental Toxicology which is a separate technical report (AFAMRL-TR-82-101).

Our next conference, currently in the development stage, will be held in November 1983 at the Daytonian Hilton Hotel, Dayton, Ohio.

SECTION II
RESEARCH PROGRAM

Toxicology research conducted by the THRU during the past was primarily concerned with continuing studies of toxic and tumorigenic effects of chronically inhaled fuels. Animals exposed to high altitude aircraft fuels JP-7 and JP-TS and to a torpedo fuel, Otto Fuel II, completed a 1-year postexposure observation period and tissues were collected for histopathologic examination. Final reports of the results of these studies will await completion of the tissue examination. Subchronic inhalation studies of 21 and 90 day duration were conducted on three hydraulic fluids and 90 day continuous exposures of animals to JP-8 were performed during the past year.

The JP-8 exposed animals are currently undergoing postexposure observation for comparison with results of other aircraft fuel exposures.

Other research activities of the THRU during the past year included a series of acute toxicity tests and the chemical characterization of aerosols of O-ethyl(2-diisopropyl-aminoethyl)-O'-methylphosphonite (EDMP). Additional hydraulic fluids of various chemical composition were studied for acute toxic effects.

The current status of these ongoing studies is summarized in this report.

EVALUATION OF THE ONCOGENIC POTENTIAL OF INHALED HYDRAZINE IN RATS AND HAMSTERS AFTER A SERIES OF WEEKLY ONE-HOUR EXPOSURES

One of the important uses of the strategic missile fuel, hydrazine, is as a fuel in standby power systems of operational aircraft. Maintenance of the systems may result in occasional accidental human exposure to high concentrations for brief periods. The specific concern and purpose of this study was to assess the oncogenic risk of several short high concentration exposures of maintenance personnel to hydrazine. The design and conduct of this study simulated severe intermittent human exposure utilizing the total doses of hydrazine that had caused pulmonary tumors and nasal polyps in rats and hamsters in previous chronic inhalation exposure studies.

Background

Hydrazine was shown to be a weak oncogen in rats and hamsters exposed to 5.0 ppm and in rats and mice exposed to 1.0 ppm hydrazine 6 hours/day, 5 days/week for a one-year period (MacEwen et al., 1981). The calculated dose equivalent values on CT (concentration x time) for these exposures was 7500 ppm-hours. In order to closely simulate possible accidental human exposure, the present study utilized exposure periods of one hour at the maximum nonlethal concentration for repeated exposure to hydrazine. Since the tumors induced by hydrazine were only seen in the respiratory system where direct contact occurred and were always associated with other lesions produced by the irritative effects of hydrazine on nasal epithelial surfaces, we believe that the compressed exposure of 7500 ppm hours is a suitable test for the comparison of short versus

long-term exposure at the same CT values. Single weekly one-hour exposures permitted recovery from the acute effects of hydrazine before subsequent exposure challenges. Sufficient numbers of one-hour exposures to the maximum nonlethal concentrations were utilized to reach a CT of 7500 ppm-hours. Rats and hamsters were selected as the test species since a 7500 ppm-hour CT of hydrazine has already been demonstrated to produce nasal tumors in each of these species.

Methods

A brief description of the protocol for preliminary studies was presented in the 1980 THRU Annual Report (MacEwen and Vernot, 1980). A complete description of the protocol along with the findings of the Phase I and Phase II portions as well as the Phase III exposure data through May, 1981 was presented in the 1981 THRU Annual Report (MacEwen and Vernot, 1981). A review of body weight data and mortality was presented in the 1982 THRU Annual Report (MacEwen and Vernot, 1982).

Phase I was designed as a range finding study to determine the one-hour LC_{50} values for male and female rats. Preliminary exposures demonstrated, however, that it was impossible to generate sufficiently high vapor concentrations of hydrazine for LC_{50} determinations without aerosol formation. Nevertheless, preliminary exposures indicated the maximum nonlethal level was approximately 750 ppm for repeated 1-hour exposures. The experimental approach was, therefore, modified so that Phase I consisted of exposing 10 male rats, 10 female rats, and 20 male hamsters to a concentration of 750 ppm hydrazine twice per week for 5 weeks.

The 10 exposures were conducted in a 1 m^3 Rochester Chamber. The chamber concentration of 750 ppm hydrazine was first established and stabilized. The rats and hamsters in groups of 10 were then rapidly inserted into the chamber by means of sliding cage drawers. At the end of one hour the animals were rapidly removed. A total of 4 cage drawers were used.

In the absence of a nonexposed control group, statistical evaluation of the data was not conducted. However, even in the absence of statistical comparisons, it was apparent that the body weight gains of all animal groups exposed to the 750 ppm concentration of hydrazine were adversely affected over the entire exposure period. By the 10th exposure, weight loss was seen for male rats and hamsters while female rats showed minimal weight gains. Recovery was seen for all groups by 2-weeks postexposure. The stress of exposure

was reflected in a general unthrifty appearance of the animals, but there was no mortality in any group.

Phase II exposures were conducted in the same manner (sliding cage drawers) and utilized the same chamber as Phase I. Slightly younger animals were used and matched chamber control groups were included. The 1-hour exposures were conducted once a week. Ten male rats, 10 female rats, and 20 hamsters as well as equivalent numbers of controls were utilized. From these groups 5 male rats, 5 female rats, 10 hamsters and an equal number of controls were killed after the first 1-hour exposure for gross and histologic examination. The remaining animals were killed and examined 24 hours after the final exposure.

The results of Phase II served adequately as a pilot study for Phase III in that it demonstrated that repeated weekly one-hour exposures to 750 ppm hydrazine were tolerated by rats and hamsters with no mortality. A total of 900 rodents were used in Phase III to evaluate the oncogenic potential of hydrazine following exposure to the selected concentrations of 750 or 75 ppm. The latter dose was chosen in an attempt to establish a no-effect level. The exposure regimen was the same as established in Phase II: one hour a week for 10 weeks (total CT values 7500 and 750 ppm hours). The two exposure groups as well as an unexposed control group each consisted of 100 male rats, 100 female rats, and 100 male hamsters.

Results

Body weight data for Phase III male and female rats and for male hamsters are presented in Figures 1, 2, and 3, respectively. The mean body weight values of male rats from the 3 treatment groups have been essentially equal during the majority of the past year. However, body weights have begun to decline and this decline has been slightly more rapid in the 750 and 7500 CT treatment groups. The difference between the control and 7500 CT group was significant at the .05 level of significance at week 102 postexposure. The female rat mean body weight values of the control rat group have been slightly greater than weights of rats from both treatment groups for the majority of the past year. However, at 102 weeks postexposure all groups were essentially equal. The hamster body weight trends discussed in 1981 THRU Annual Report were consistent until their removal from the experiment at 22 months postexposure. At that time the hamsters were approximately 30 months of age. The control hamster body weights were lower than those of hydrazine exposed hamsters throughout the study. The hamsters were necropsied and tissues taken for histopathology examination of lesions.

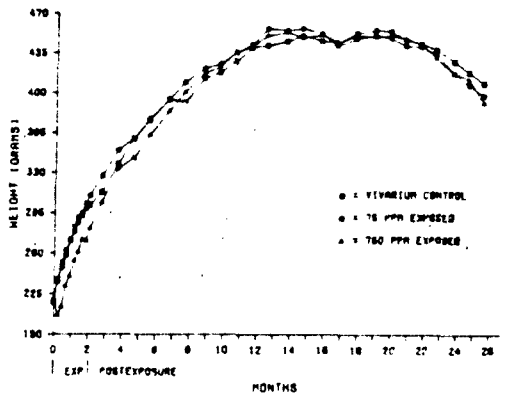


Figure 1. Effect of Phase III hydrazine exposure on male rat body weight.

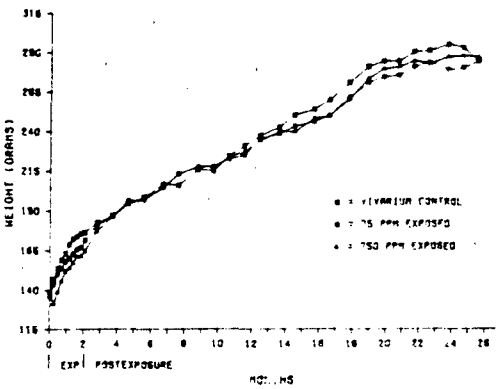


Figure 2. Effect of Phase III hydrazine exposure on female rat body weight.

Current mortality data for male and female rats are presented in Table 1. Seven male and female rats of each group were sacrificed at 30 months of age. The results of histologic examination of tissues taken at this time will be reported in future annual reports. The mortality ratios indicate no unexpected trends.

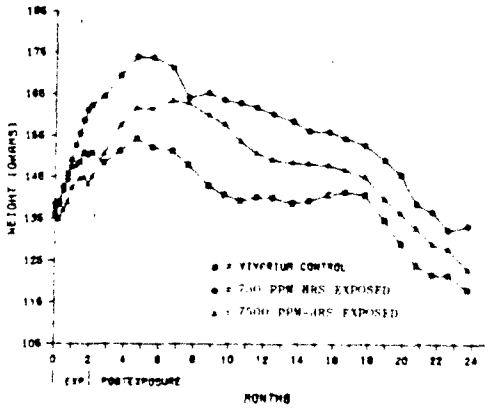


Figure 3. Effect of Phase III hydrazine exposure on male hamster body weight.

TABLE 1. MORTALITY IN FISCHER 344 RATS 2 YEARS POSTEXPOSURE TO BRIEF HIGH LEVELS OF INHALED HYDRAZINE (7500 PPM-Hours)

Sex	Nominal CT (ppm/hours)	Mortality Ratio
Male	0	53/93
	750	49/93
	7500	46/93
Female	0	29/93
	750	45/93
	7500	33/93

Hamster mortality up to the time of termination of the study is shown in Figure 4. At termination, the mortality rates of the two treatment groups were equal and still less than that of the control group.

The final necropsy of male and female rats is scheduled for August 1983, at which time they will have been held for 30 months following the first hydrazine exposure. Further information will be reported when the results of tissue examinations are complete and reviewed. The results to date are similar to those observed when rats and mice were presented daily industrial type exposures to low levels of inhaled hydrazine, i.e., there are no apparent alterations in morbidity or mortality.

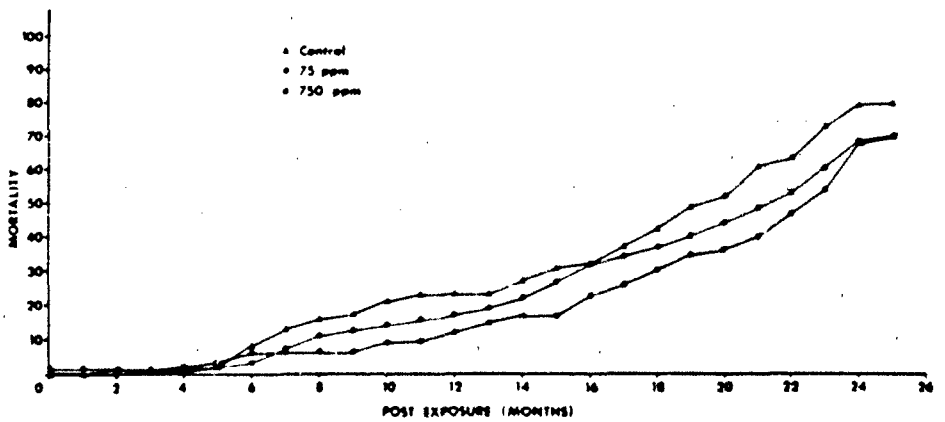


Figure 4. Cumulative mortality of hamsters exposed to hydrazine in Phase III.

THE EXPERIMENTAL DETERMINATION OF SAFE ATMOSPHERIC EXPOSURE CONCENTRATIONS OF JP-10 JET FUEL

Introduction

JP-10 is a synthetic saturated polycyclic hydrocarbon. It has been utilized as a jet fuel either alone or as a major constituent (70%) of JP-9 fuel because of its high density and other desirable properties. In the latter application, it has been substituted for RJ-4, a reduced dimer of methylcyclopentadiene. It is also used as a missile propellant.

JP-10 is a single chemical entity identified as tricyclo $(5.2.1.0^{2,6})$ decane. It is the exo isomer of tetrahydrodicyclopentadiene. Gas chromatographic analysis of this fuel indicated that it has a 98% purity. The endo isomer of tetrahydrodicyclopentadiene is the major impurity (about 2%). A detailed review of the chemistry and the use of JP-10 was presented at the Thirteenth Conference on Environmental Toxicology (1982) by Inman.

The known physical properties of JP-10 are shown below:

Molecular weight:	136
Boiling point:	360°F
Density, 70°F:	0.940
Viscosity, 70°F:	3.5
Flash point:	135°F
Saturated vapor concentration:	~1500 ppm

Since no information appeared in the literature concerning the toxicologic properties of JP-10, a series of studies was planned beginning with acute studies, including experiments for determination of emergency exposure limits and culminating in a long-term chronic study. The experiments were conducted to develop the necessary data for hazard evaluation and establishment of safe exposure limits as well as to identify the oncogenic potential of JP-10 fuel.

Preliminary acute inhalation experiments had shown that mice were the most sensitive species to JP-10 when 6 animals exposed to 1000 ppm died within 4 hours. To aid in selection of a concentration of JP-10 suitable for use in a year-long, 6 hours/day, 5 days/week exposure regimen, groups of 5 female rats and 5 female mice were exposed to 250 ppm for five 6-hour exposure days. The coordination of the mice appeared slightly affected on the first day of exposure. Respiration rates of both rats and mice were more rapid than normal during the second days' exposure. One mouse had a slight convulsion early on the second exposure day, but recovered and appeared normal thereafter. For the rest of the exposure, no further signs of toxic stress were noted in either species. Mean body weight of the mice did not increase during the week following termination of exposure. A detailed report of all acute inhalation experiments, including emergency limit studies, oral and intraperitoneal toxicity, dermal and ocular irritation tests, and sensitization studies, can be found in previous annual reports (MacEwen and Vernot, 1979, 1980).

As a result of the toxic effects shown in mice in the short-term inhalation tests, a concentration of 100 ppm (556 mg/m^3) JP-10 was selected for chronic inhalation studies with animals to determine safe exposure limits. Following the inception of this experiment in 1978, other researchers have conducted and reported results of JP-10 acute toxicity studies (Burdette, 1978; Keller, 1981; Lyng, 1981; Inman, 1982).

Materials and Methods

Each exposure chamber contained as few species as possible to minimize the risk of cross infection. Therefore, dogs and rats were housed in one chamber and mice and hamsters in a companion chamber. The numbers of animals in each chamber and cage were compatible with ILAR standards for animal care. The numbers of rodents permitted a statistically valid number of each species to reach the required age for tumor induction with natural and toxicologic attrition. Purebred beagle dogs were selected from a baseline group on the basis of examination and general observation of good health and several preexposure clinical chemistry determinations. Fischer 344 rats and Golden Syrian hamsters were obtained from the Charles River Breeding Laboratories. C57BL/6 mice were purchased from the Jackson Laboratory. Distribution of the animal groups and other pertinent information is shown in Table 2.

TABLE 2. SPECIES, SEX, STRAIN, AND NUMBER OF ANIMALS EXPOSED TO JP-10 VAPORS FOR 12 MONTHS

			100 PPM (556 MG/M^3) JP-10		Unexposed
Species	Sex	Strain	Chamber 1	Chamber 2	Controls
Rats	M	Fischer 344	---	50	50
Rats	F	Fischer 344	---	50	50
Mice	F	C57BL/6	200	---	200
Hamsters	M	Golden Syrian	100	---	100
Dogs	M	Beagle	---	4	4
Dogs	F	Beagle	---	4	4

All animals were observed hourly during the exposure phase of the study. Daily observations were conducted during the postexposure phase until termination of the experiment. Exposure to JP-10 was for one year, using an industrial work week schedule of 6 hours/day, 5 days/week, with holidays and weekends off to simulate a human exposure regimen.

Food was provided to the animals during the nonexposure times, and the chambers were cleaned daily following the completion of the 6-hour exposure and minimum 30-minute air purge. Analysis of chamber concentration was used to verify the adequacy of the purge time.

Rats, hamsters, and dogs were weighed individually at biweekly intervals during exposure and weighed monthly during the postexposure period. Mice were weighed in groups with group mean

weights followed on a monthly basis throughout the experimental period.

Blood samples were drawn from all dogs at biweekly intervals for determination of the following tests:

HCT	Potassium
HGB	Calcium
RBC	Albumin/Globulin
WBC	Total Protein
Differential Cell Counts	Glucose
MCV	Alkaline Phosphatase
MCH	SGPT
MCHC	SGOT
Sodium	Bilirubin
BUN	Creatinine

Following the one-year exposure period, 20 mice/group and 10 hamsters/group were necropsied to determine chronic exposure effects, while the remaining rodents were held for a year of post-exposure observation. The dogs are being held for postexposure observation for five years, during which time they receive quarterly physical examinations and semi-annual blood analysis. The dogs are scheduled to be killed and examined in June, 1984.

Gross and histopathologic examinations were conducted on all animals that died or were sacrificed during and at the completion of the study. The necropsy protocol was an external examination, including body orifices, and examination and fixation of 33 tissues using the NCI protocol for oncogenic screening. Autolysis or cannibalization prevented partial or complete examination in some cases. Tables of selected tumor and non-tumor incidence were compiled and statistical analysis using the Fisher Exact Test was performed by the UCI staff.

The exposure chambers were operated with nominal airflows of 30 cfm at a slightly reduced pressure, 725 mm Hg, to avoid leakage of JP-10 vapor into the laboratory environment.

The JP-10 used for these animal exposures was obtained by the Air Force from Suntech, Inc., Marcus Hook, Pennsylvania. A Buchler Polystaltic® Pump was used to deliver the liquid JP-10 from a storage drum into a spiral evaporator where it was vaporized and introduced into the chamber air supply system through a 1/4" stainless steel line. The exposure dome concentrations were monitored using a Beckman Model 400 Hydrocarbon Analyzer. Sequential sampling was conducted on the pair of chambers. The supply of JP-10 in use

was analyzed once per month during the study, and each time a sample drum was changed. This was done using a Varian Model 3700 gas chromatograph with a 1/8" x 10' stainless steel column containing 10% SE 30 on Chromosorb W and helium carrier gas at 30 ml/min. The column was temperature programmed from 90°C to 200°C, at 10° per minute. The detector and injector temperatures were 220°C and the injection volume was one microliter.

Results

The nominal desired concentration of 100 ppm JP-10 was maintained in both chambers with little variation between the chambers throughout the 12-month exposure phase of the experiment (June 5, 1978 through June 4, 1979). As shown in Table 3, monthly mean concentrations never exceeded 1.4% or were less than 0.8% of the desired JP-10 concentration. Overall mean concentrations for Chambers 1 and 2 respectively were only 0.4% and 0.2% lower than the 100 ppm desired concentration.

TABLE 3. MONTHLY JP-10 CONCENTRATIONS (PPM) THROUGH THE 12-MONTH EXPOSURE PERIOD (MEAN ± STANDARD ERROR)

Month	Chamber 1	Chamber 2
June, 1978	99.2 ± 0.28	100.0 ± 0.08
July, 1978	99.4 ± 0.20	99.5 ± 0.30
August, 1978	100.8 ± 0.10	98.9 ± 0.14
September, 1978	101.4 ± 0.08	101.1 ± 0.10
October, 1978	100.3 ± 0.12	100.3 ± 0.10
November, 1978	99.3 ± 0.07	100.0 ± 0.09
December, 1978	99.4 ± 0.08	99.7 ± 0.06
January, 1979	99.8 ± 0.08	100.5 ± 0.08
February, 1979	99.6 ± 0.05	99.7 ± 0.09
March, 1979	100.4 ± 0.06	99.9 ± 0.04
April, 1979	99.9 ± 0.12	100.4 ± 0.11
May, 1979	100.2 ± 0.11	99.7 ± 0.08
June, 1979	99.7 ± 0.23	100.0 ± 0.25
Overall Mean	99.6	99.8

The mortality ratios at the end of the 12 months of exposure and at 12 months postexposure immediately prior to the sacrifice of all surviving rodents in June, 1980, are shown in Table 4. This table includes the 20 exposed mice, 20 control mice, 10 exposed

hamsters and 10 control hamsters killed at the end of exposure and submitted for necropsy to determine if tissue changes were present at that time. There was no effect of JP-10 exposure on rodent mortality. One female control dog died in July, 1981. This animal had demonstrated several epileptic type seizures, several times.

TABLE 4. MORTALITY RATIOS FOR GROUPS OF JP-10 EXPOSED AND CONTROL ANIMALS AT EXPOSURE CONCLUSION AND AT 12 MONTHS POSTEXPOSURE

		Unexposed Controls		100 ppm JP-10 Exposed	
Species	Sex	Exposure Conclusion	12-Months Postexposure	Exposure Conclusion	12-Months Postexposure
Mice	F	30/200	[a]148/200	20/200	[a]143/200
Rats	M	0/50	16/50	0/50	8/50
Rats	F	4/50	21/50	0/50	20/50
Hamsters	M	5/100	[a] 47/100	9/100	[a] 43/100
Dogs	M	0/4	0/4	0/4	0/4
Dogs	F	0/4	0/4	0/4	0/4

[a]Includes 10% sacrificed within 2 days of exposure conclusion.

Mean body weights for groups of exposed and control male rats, female rats, and male hamsters, obtained on a biweekly schedule throughout 12 months of exposure and monthly through 12 months postexposure, are shown in Figure 5. Weights of male rats and hamsters showed depression relative to controls as a result of JP-10 exposure. Values for male rats were statistically different from control values at all times during exposure and postexposure. Values for exposed hamsters were also statistically different from controls at all weighing periods during exposure but not during the postexposure phase of the experiment. Exposed female rat weights were not significantly different from controls at any phase of the study. An examination of all mean weights of dogs and mice taken during and after exposure revealed no effect of JP-10 exposure.

An examination of the hematology and clinical chemistry values from the battery of tests conducted biweekly on dogs throughout the 52 weeks of exposure revealed nothing noteworthy except for total protein and globulin results. Albumin values for exposed dogs were stable and comparable with controls throughout the exposure. Slightly elevated protein values, therefore, were reflected in slight elevations in the calculated globulin fraction from weeks 2 through 52. Statistical differences from control values were seen

in 9 of 16 measurements from exposure week 22 to exposure conclusion, but the albumin/globulin ratios for the exposed dogs were well within normal limits for this species. No toxicologic significance is attached to this finding. The results of postexposure, quarterly physical examinations, and semi-annual clinical chemistry measurements indicate that all exposed and control dogs are in good health. The 15 surviving dogs will be maintained until June, 1984.

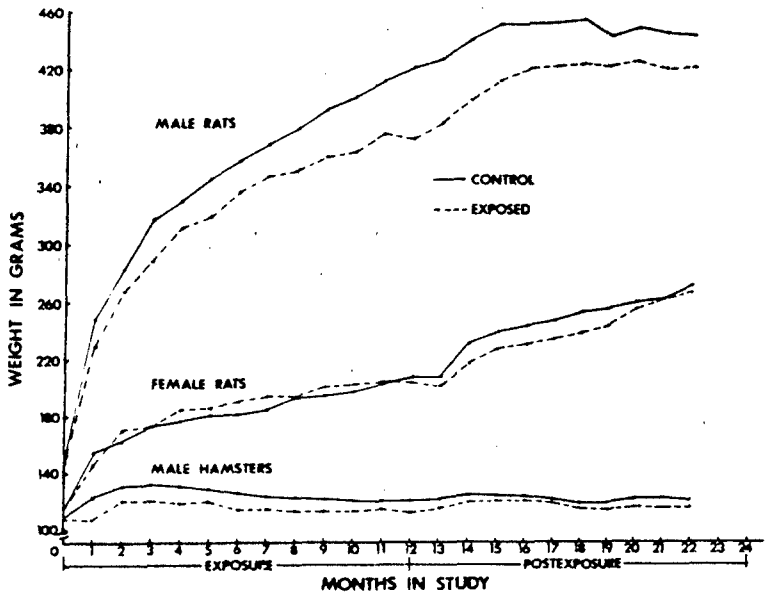


Figure 5. Mean body weight of rats and hamsters exposed intermittently to 100 ppm JP-10 for one year.

As mentioned previously, one female control dog died in July, 1981, approximately three years after the start of the experiment. This animal had a record of epileptic seizures, but microscopic examination of tissues failed to reveal the cause of death. CNS lesions were not observed grossly or microscopically.

Preliminary examination of the numbers of various non-neoplastic lesions in mice that died during the exposure phase of the study compared with the numbers of lesions in mice that were killed at exposure conclusion failed to reveal any difference that could be attributed to duration of JP-10 exposure. Therefore, lesion

incidences were combined and expressed as lesions - through 1 year of exposure in Table 5. This table also shows non-neoplastic lesions in mice through one year postexposure.

TABLE 5. NON-NEOPLASTIC LESIONS[a] IN FEMALE C57BL/6 MICE EXPOSED TO JP-10

	Through 1 Year of Exposure		Through 1 Year Postexposure	
Lesion Description	Unexposed Controls	100 ppm Exposed	Unexposed Controls	100 ppm Exposed
Skin	N=48	N=31	N=149	N=157
Inflammation (Ulcerative Dermatitis)	16(33)	0(0)[b]	21(14)	29(19)
Nose	N=46	N=30	N=146	N=153
Hyaline Degeneration, Crystals	21(46)	2(7)[b]	85(58)	66(43)[b]
Lung	N=46	N=28	N=148	N=155
Hyaline Degeneration, Crystals	0(0)	0(0)	18(12)	39(25)[b]
Alveolar Macrophages	1(2)	1(4)	16(11)	20(13)
Perivascular Cuffing	2(4)	4(14)	17(12)	19(12)
Bone Marrow	N=45	N=29	N=137	N=143
Granulocytic Hyperplasia	22(49)	9(31)	55(40)	81(57)[b]
Spleen	N=44	N=27	N=142	N=148
Granulocytic Hyperplasia	15(34)	4(15)	29(20)	30(20)
Liver	N=46	N=28	N=149	N=155
Fatty Change	31(67)	21(75)	70(47)	95(61)[b]
Gallbladder	N=40	N=24	N=122	N=124
Hyaline Degeneration	5(13)	5(21)	11(9)	29(24)[b]
Kidney	N=46	N=28	N=150	N=153
Glomerulonephritis	0(0)	3(11)	18(12)	21(14)
Uterus	N=43	N=26	N=148	N=153
Cysts	0(0)	0(0)	1(1)	21(14)[b]
Ovary	N=40	N=24	N=137	N=143
Cysts	0(0)	1(4)	23(18)	40(29)[c]
Thyroid	N=42	N=25	N=137	N=43
Follicle, Papillary Hyperplasia	0(0)	1(4)	100(73)	86(60)[c]

N=Number Examined

()=Percent Incidence

[a]Number of lesions/numbers of animals examined.

[b]Statistically different from control incidence at $p \leq 0.01$.

[c]Statistically different from control incidence at $p \leq 0.05$.

Severe ulcerative dermatitis was observed in 33% of the control mice, whereas no skin lesions were seen in exposed mice through one year of exposure. This is a common finding in C57BL/6 mice, but the high incidence in the controls may be the reason that more of them died during the first year of the experiment (30 controls versus 20 exposed). There is a higher incidence of nasal crystals, bone marrow and splenic granulocytic hyperplasia in controls compared with exposed mice through one year of exposure. The frequency of ulcerative dermatitis was nearly equal in exposed and control mice that died or were killed during or at the end of the postexposure period. The incidences of gall bladder, lung crystals, and bone marrow hyperplasia are significantly higher in exposed mice than in controls. All of these findings may be related to the observed skin disease.

High incidences of fatty livers were found in both the exposed and control mice. The incidence was greater in exposed mice than in controls, but without statistical significance, through one year of exposure. A statistical difference was measured for exposed mice after one year postexposure. The incidence of fatty liver in exposed and control mice decreased with age and duration of the experiment, providing evidence that this lesion was not a result of aging and that the excess of fatty livers in exposed mice was due to JP-10 toxicity.

Ovarian and uterine cysts are extremely common in aged female mice, but a statistically significant increase of both lesions is seen for exposed mice through one year postexposure. This apparent increase of naturally occurring, age-related diseases should not be ruled out in the consideration of the toxicity of JP-10. Papillary hyperplasia of thyroid follicular epithelium was a frequent finding in mice through one year postexposure. However, the incidence was greater in the unexposed control group.

The incidences of neoplastic lesions in exposed and control mice were similar. The majority of tumors occurred with equally low frequency in both groups. There was a large number of pituitary adenomas and lymphomas of multiple organs in all mice through the one-year postexposure period. Statistical calculations failed to reveal significant differences in the incidence of these tumors between exposed and control animals.

Non-neoplastic lesions found in hamsters are shown in Table 6. During exposure, the only lesion more prevalent in exposed animals was congestion of the lungs. This is a common finding, however, and may have resulted from non-pathologic events, such as postmortem vascular stasis. In exposed hamsters maintained through

one year exposure, there is a statistical excess of hyperplastic lesions of the adrenal cortex. The adrenal hyperplasia was most often seen in the zona glomerulosa (outer zone) as opposed to the other zones of the cortex with a statistically significant increase in the exposed hamsters. Additional findings in exposed hamsters included a significant incidence of testicular and pancreatic atrophy, which may represent an accelerating effect of JP-10 exposure on the aging process. Noteworthy, but without statistical support, is the increased incidence of fatty livers in the exposed hamsters compared with controls.

TABLE 6. NON-NEOPLASTIC LESIONS[a] IN MALE GOLDEN SYRIAN HAMSTERS EXPOSED TO JP-10

	Through 1 Year of Exposure		Through 1 Year Postexposure	
Lesion Description	Unexposed Controls	100 ppm Exposed	Unexposed Controls	100 ppm Exposed
Lung	N=15	N=17	N=84	N=78
Congestion	6(40)	12(71)	9(11)	6(8)
Liver	N=14	N=17	N=84	N=79
Fatty Change	0(0)	0(0)	39(46)	44(56)
Pancreas	N=10	N=14	N=75	N=68
Atrophy	1(10)	0(0)	0(0)	6(9)[b]
Testis	N=15	N=18	N=84	N=79
Atrophy	0(0)	0(0)	12(14)	28(35)[c]
Prostate	N=11	N=15	N=77	N=65
Atrophy	0(0)	0(0)	6(8)	8(12)
Seminal Vesicle	----	----	N=81	N=77
Atrophy	----	----	2(3)	6(8)
Adrenal Cortex	N=15	N=17	N=85	N=78
Hyperplasia	2(13)	3(18)	38(45)	67(86)[c]

N=Number Examined
()=Percent Incidence
[a]=Number of lesions/number of animals examined.
[b]=Statistically different from control incidence at $p \leq 0.05$.
[c]=Statistically different from control incidence at $p \leq 0.01$.

The only neoplastic lesions of interest noted in hamsters are listed in Table 7. Adrenocortical tumors were found in both the exposed and control hamsters through one year postexposure. The combined incidence of cortical adenomas and carcinomas was 27% in controls and 28% in exposed subjects. Of this group, however, 11 of the exposed animals exhibited adenomas of the zona glomerulosa, whereas only 3 of the control hamsters had tumors of the outer zone of cortical cells. This finding, combined with the observation of substantially increased hyperplasia of the zona glomerulosa in exposed subjects, as mentioned previously, may indicate an effect of JP-10 on the mineralocorticoid-secreting cells of the adrenal gland. However, in a review of spontaneous and non-viral induced neoplasms by Kirkman and Algard (1968) the combined incidence of adenomas and carcinomas found in 4,575 untreated control hamsters was 35.5% for the second year of life and the vast majority of these lesions were found in the zona glomerulosa. There were no other significant JP-10 exposure-related increases in neoplasms. It is emphasized that no primary pulmonary or hepatic tumors were found in exposed animals. An hepatocellular adenoma was observed in one control hamster.

TABLE 7. NEOPLASTIC LESIONS[a] IN THE ADRENAL CORTEX OF MALE GOLDEN SYRIAN HAMSTERS EXPOSED TO JP-10

	Through 1 Year of Exposure		Through 1 Year Postexposure	
Tumor Type	Unexposed Controls	100 ppm Exposed	Unexposed Controls	100 ppm Exposed
Adrenal Cortex	N=15	N=17	N=85	N=78
Adenoma	0(0)	1(6)	12(14)	15(19)
Carcinoma	0(0)	0(0)	11(13)	7(9)
Total	0(0)	1(6)	23(27)	22(28)

N=Number Examined
()=Percent Incidence
[a]=Number of lesions/number of animals examined.

Table 8 lists non-neoplastic lesions seen in female and male rats following one year of exposure to JP-10. Various lesions indicative of accentuated renal tubular nephrosis in exposed male rats are in strong evidence. Accentuated renal tubular degeneration, medullary mineralization, and papillary hyperplasia of the

TABLE 8. NON-NEOPLASTIC LESIONS[a] IN FEMALE AND MALE FISCHER 344 RATS FOLLOWING EXPOSURE TO JP-10

	FEMALES		MALES	
Lesion Description	Unexposed Controls	100 ppm Exposed	Unexposed Controls	100 ppm Exposed
Spleen	N=47	N=49	N=50	N=49
Hemosiderosis	13(28)	25(51)[b]	6(12)	11(22)
Heart	N=49	N=50	N=50	N=50
Myocardium Fibrosis	5(10)	7(14)	26(52)	32(64)
Blood Vessels	N=50	N=50	N=50	N=50
Pulmonary Artery Mineralization	12(24)	20(40)	24(48)	17(34)
Liver	N=49	N=49	N=49	N=50
Fatty Change	7(14)	10(20)	1(2)	4(8)
Focal Cellular Change	(26(53)	27(55)	37(76)	40(80)
Bile Duct Hyperplasia	18(37)	22(45)	45(92)	49(98)
Kidney	N=50	N=50	N=49	N=49
Tubular Pigmentation	4(8)	17(34)[c]	4(8)	5(10)
Medullar Mineralization	0(0)	0(0)	0(0)	49(100)[c]
Pelvic Papillary Hyperplasia	0(0)	0(0)	2(4)	43(88)[c]
Tubular Degeneration	1(2)	2(4)	32(65)	43(88)[c]
Adrenal	N=49	N=49	N=50	N=50
Focal Cellular Change	18(37)	13(27)	13(26)	17(34)

N=Number Examined
()=Percent Incidence
[a]=Number of lesions/number of animals examined.
[b]=Statistically different from control incidence at $p \leq 0.05$.
[c]=Statistically different from control incidence at $p \leq 0.01$.

pelvic transitional epithelium were observed with significantly increased frequency or severity in exposed males, and were considered to be the extension of a toxic tubular nephrosis which occurred during the exposure phase. These lesions were entirely consistent with histopathologic findings in male rats exposed subchronically (90 day continuous) to other nephrotoxic hydrocarbons and held for long-term evaluations (MacEwen and Vernot, 1978, 1979, 1980, 1981, and 1982). Marked medullary mineral deposits were present in all exposed males but were entirely absent from controls. These deposits were regarded as mineralized cell debris which originated from toxic necrosis of proximal tubular epithelium. Papillary hyperplasia of the renal pelvis was noted in 26/49 (53%) of the exposed and only 2/49 (4%) of the controls. The pathogenesis of this lesion remains obscure but may be related to mechanical forces such as increased intrapelvic friction or the abrasive action of mineralized concretions shed from renal tubules. Accentuated and more frequent tubular degeneration was documented in exposed males where 43/49 (88%) exhibited minimal to moderate tubular degeneration as compared with 32/49 (65%) of the controls where changes were graded as only minimal to mild. On a severity scale of 1 to 4, where 4 is the most severe, tubular degeneration was graded as 1.8 in the exposed rats and only 1.3 in the controls. Since many of the morphologic features of accentuated tubular degeneration were entirely compatible with spontaneous, chronic progressive nephrosis of old rats, frequency and severity data are important in establishing a relationship with JP-10 exposure. Bruner (1982) has described in detail the pathology of nephrotoxicity of hydrocarbons in male rats in a number of experiments conducted in our laboratory in a presentation at the Thirteenth Conference on Environmental Toxicology.

There were statistically significant differences in the incidences of spleen and kidney lesions in female exposed rats, compared with controls. Minimal to mild splenic hemosiderosis was present in 51% of the exposed females, while only 28% of the controls showed increased hemosiderin deposits. This is a highly subjective diagnosis and its etiology and pathologic significance remain uncertain. Renal tubular cytoplasmic lipochrome pigmentation was observed in 34% of the exposed animals and in 8% of the control group. The genesis of this common renal pigment and its increased frequency in exposed female rats is not certain. It could be associated with mononuclear cell leukemia and the related compromise of hepatic and hematologic parameters and functions.

Neoplasms seen in male and female exposed and control rats following the one year postexposure period are shown in Table 9. Mononuclear cell leukemia was present in 22% of the exposed females and in 4% of the controls. Although the distinct increase in exposed females implies an effect of JP-10 exposure, it is generally agreed that mononuclear cell leukemia is very common in aging Fischer 344 rats. Therefore, it is most likely that this event is not treatment related.

TABLE 9. NEOPLASTIC LESIONS[a] IN FEMALE AND MALE FISCHER 344 RATS FOLLOWING EXPOSURE TO JP-10

	FEMALES		MALES	
Tumor Type	Unexposed Controls	100 ppm Exposed	Unexposed Controls	100 ppm Exposed
Kidney	N=50	N=50	N=49	N=49
Carcinoma	0(0)	0(0)	1(2)	4(8)
Adenoma	0(0)	0(0)	(0)	5(10)[c]
Testes	----	----	N=50	N=49
Interstitial Cell Tumor	----	----	44(88)	47(96)
Pituitary	N=47	N=46	N=50	N=49
Adenoma	15(32)	15(33)	15(33)	11(23)
Pancreas	N=47	N=47	N=45	N=48
Islet Cell Adenoma	0(0)	3(6)	1(2)	0(0)
Multiple Organs	N=50	N=50	N=50	N=50
Mononuclear Cell Leukemia	2(4)	11(22)[b]	6(12)	5(10)

N=Number examined.
()Percent Incidence.
[a]Number of lesions/number of animals examined.
[b]Statistically different from control incidence at $p \leq 0.01$.
[c]Statistically different from control incidence at $p \leq 0.05$.

Although it lacks statistical significance, the most toxicologically significant finding in male rats was the presence of four renal cell carcinomas in the kidneys of exposed rats as compared with one renal cell tumor in controls. After completion of the examination of the tissues, information was received that hydrocarbon induced tumors were most commonly seen in the polar region of the kidney. Our protocol for examination of the kidney did not include a section through the polar region and therefore additional sections of renal tissue were examined. In addition to the four primary renal tumors which were observed during the intial study of these tissues, five more small renal cell adenomas were discovered in polar zones of retrimmed kidney specimens. The incidences after reexamination are included in Table 9. No primary renal cell tumors were observed in retrimmed control tissues. Statistical comparison of the incidence of adenomas in male exposed and control rats produced a difference at $p \leq 0.05$. Total tumors in exposed male rats were also statistically greater than in the control group - nine in exposed versus one in controls. These findings strongly suggest that chronic exposure to JP-10 vapors causes malignant and nonmalignant renal cell tumors in male rats. The total tumor incidence of 18% is considerably higher than the naturally occurring incidence of primary kidney tumors in male Fischer 344 rats of 0.5%, as tabulated in National Toxicology Program statistics (Chu et al., 1981).

Summary and Conclusion

In summary, the outstanding effects of repeated exposure to 100 ppm JP-10 were fatty livers in mice and renal tubular nephrosis together with a significant increase in malignant and nonmalignant renal cell tumors in male rats.

The results of this study provide evidence that 100 ppm JP-10 may not be a safe exposure level for man. Based on the information from this study, a TWA of 25 ppm JP-10 has been recommended.

A SUBCHRONIC TOXICITY STUDY OF 90 DAY CONTINUOUS EXPOSURE TO SHALE JP-5

A 90 day continuous inhalation toxicity study of oil Shale derived JP-5 jet fuel was conducted in 1979 to determine if the health hazards of oil Shale materials differ from those associated with petroleum derived products. The protocol, contaminant,

generation and monitoring methods used for the study were similar to other 90 day continuous inhalation studies and were detailed in a previous annual report (MacEwen and Vernot, 1980).

Groups of 3 male and female beagle dogs, 75 male and female Fischer 344 rats, and 150 female C57BL/6 mice were continuously exposed to concentrations of 150 mg/m^3 or 750 mg/m^3 Shale JP-5 vapor in Thomas Dome inhalation chambers. Unexposed controls were held in laminar air flow rooms in separate facilities. At the conclusion of the exposure, all dogs and 1/3 of the rodents were killed for gross and histopathologic tissue examination to detect any pathologic lesions caused by exposure to Shale JP-5. This examination occurred in October 1979.

The remaining rodents were held for postexposure observation for 19 months, at which time one-half of the animals were killed for tissue collection and examination. The remaining animals were held until the 24th month of the study at which time they were killed for tissue comparison with unexposed controls and with petroleum JP-5 exposed animals. Results of this study have been presented as they became available in the 1980, 1981 and 1982 annual reports (MacEwen and Vernot). During this current report period the results of tissue examination of the Fischer 344 rats exposed to vapors of Shale JP-5 and their unexposed controls became available.

A dose related depression in male rat body weight gain was evident throughout the entire study period. Depressed weight gain in female rats was seen only in the 750 mg/m^3 exposure group. Other signs of Shale JP-5 toxicity included increased kidney weight and elevated BUN and creatinine levels in male rats necropsied at the termination of exposure. Shale JP-5 related tissue changes included nasal inflammation and hepatocyte cytoplasmic vacuolization in male and female rats, renal tubular necrosis in almost all male rats and fatty livers in female mice.

The majority of lesions seen in the Shale JP-5 exposed rats were common to aged rats and were unrelated to the inhalation exposure to this hydrocarbon fuel. Selected pathologic changes seen in male rats are shown in Table 10. The significance probabilities of the differences between all exposed and control means were calculated using the Fisher Exact Test. Renal tubular degeneration was seen frequently in all groups of male rats. This lesion is consistent with commonly occurring renal nephropathy in older male

rats. The tubular degeneration was seen more frequently and more severely in both groups of male rats exposed to Shale JP-5 when compared to controls. Moderate to severe deposits of mineralized debris were found in the tubules of exposed male rats. Accompanying the mineralization was diffuse papillary hyperplasia of the pelvic urothelium. The hyperplasia was dose related in frequency. Foci and areas of liver cell alteration diagnosed as "focal cellular change" and "clear cell change" appeared with approximately equal distribution among the three groups. Bile duct hyperplasia is a common lesion seen in older Fischer 344 rats and no indication of a JP-5 exposure effect was seen. Female rats were free of any substantial renal cell alteration (Table 11). Focal cellular change in the liver was noted slightly more frequently in exposed female rat groups compared to controls. The increase was not statistically significant at the 0.05 level of confidence, however.

TABLE 10. PATHOLOGIC CHANGES[a] SEEN IN MALE FISCHER 344 RATS HELD FOR POSTEXPOSURE OBSERVATION AFTER 90 DAY CONTINUOUS INHALATION EXPOSURE TO SHALE DERIVED JP-5

Tissue/Lesion	Unexposed Controls	150 mg/m^3 Exposed	750 mg/m^3 Exposed
Kidney			
Tubular Degeneration	34/50	44/49[b]	48/49[b]
Mineralization	0/50	49/49[b]	49/49[b]
Hyperplasia	0/50	8/49[b]	31/49[b]
Liver			
Fatty Change	3/50	3/50	4/50
Focal Cell Change	28/50	32/50	32/50
Clear Cell Change	10/50	3/50[c]	10/50
Bile Duct			
Hyperplasia	46/50	44/50	48/50

[a]Number of lesions/number of animals examined.
[b]Statistically different from control value at $p \leq 0.01$.
[c]Statistically different from control value at $p \leq 0.05$.

TABLE 11. PATHOLOGIC CHANGES[a] SEEN IN FEMALE FISCHER 344 RATS HELD FOR POSTEXPOSURE OBSERVATION AFTER 90 DAY CONTINUOUS INHALATION EXPOSURE TO SHALE DERIVED JP-5

Tissue/Lesion	Unexposed Controls	150 mg/m^3 Exposed	750 mg/m^3 Exposed
Kidney			
Tubular Degeneration	4/49	1/50	0/50
Mineralization	1/49	0/50	0/50
Liver			
Fatty Change	3/50	6/50	6/50
Focal Cellular Change	25/50	30/50	31/50
Bile Duct			
Hyperplasia	17/50	10/50	13/50

[a]Number of lesions/number of animals examined.

The majority of the tumors found in rats occurred with low incidence and were common to the species. Tumors that were seen with some frequency are shown in Tables 12 and 13 for male and female rats, respectively. Tumors were recorded in several endocrine organs in exposed rats. Increased parafollicular (C-cell) adenomas in the thyroids of exposed male rats may be related to prolonged $Ca:PO_4$ imbalances secondary to severe nephropathy. Pituitary tumors are common in aged rats and while significantly ($p \leq 0.05$) more frequent in male rats exposed to 750 mg/m^3 showed a reversed dose response in female rats. The occurrence of the adrenal medullary tumors (pheochromocytomas) was significantly higher in the male rats exposed to 750 mg/m^3 Shale JP-5 compared to controls. Female rats exposed to the higher Shale JP-5 concentration developed a slightly greater number of adrenal tumors compared to controls. However, the increases were not statistically significant at the 0.05 level of confidence.

Summary

Comparison of the results of tissue examination of rats held for long term observation after 90 days of exposure to Shale JP-5 with those of rats exposed to petroleum JP-5 (MacEwen and Vernot, 1981) fails to indicate any conspicuous difference in toxicity.

Both materials produced significant alterations in the kidneys of male rats. Renal tubular degenerative changes consistent with common progressive renal nephropathy occurred in both exposed and control groups; however, the nephropathy was considerably more severe in exposed male rats. It is assumed that the mineralized tubular deposits represent calcium impregnated cellular debris resulting from the tubular epithelial necrosis during the exposure phase of the studies.

TABLE 12. TUMORS[a] SEEN IN MALE FISCHER 344 RATS HELD FOR POSTEXPOSURE OBSERVATION AFTER 90 DAY CONTINUOUS EXPOSURE TO SHALE DERIVED JP-5

Tissue/Lesion	Unexposed Controls	150 mg/m^3 Exposed	750 mg/m^3 Exposed
Pituitary Gland			
Adenomas	5/46	9/47	13/46[b]
Carcinomas	0/46	1/47	2/46
Thyroid Gland			
Adenomas	0/50	12/50[c]	8/50[c]
Carcinomas	2/50	4/50	0/50
Testes			
Interstitial Cell Tumors	48/49	48/50	43/47
Liver			
Neoplastic Nodules	3/50	1/50	4/50
Leukemias			
All Types	12/50	8/50	11/50
Parathyroid Gland			
Adenomas	1/38	2/42	1/42
Adrenal Gland			
Phecchromocytomas	1/50	5/50	8/47[c]
Carcinomas	1/50	0/50	0/47

[a]Number of lesions/number of animals examined.
[b]Statistically different from control value at $p \leq 0.05$.
[c]Statistically different from control value at $p \leq 0.01$.

TABLE 13. TUMORS[a] SEEN IN FEMALE FISCHER 344 RATS HELD FOR POSTEXPOSURE OBSERVATION AFTER 90 DAY CONTINUOUS EXPOSURE TO SHALE DERIVED JP-5

Tissue/Lesion	Unexposed Controls	150 mg/m^3 Exposed	750 mg/m^3 Exposed
Pituitary Gland			
Adenomas	10/45	23/47[b]	11/45
Carcinomas	1/45	0/47	1/45
Thyroid Gland			
C-cell Adenomas	5/49	7/49	3/49
Uterus			
Polyps	4/47	5/50	7/48
Adenocarcinomas	2/47	6/50	0/48
Sarcoma	0/47	0/50	2/48
Hemangiomas	0/47	0/50	1/48
Clitoris			
Papillomas	2/50	0/50	1/50
Carcinomas	1/50	0/50	0/50
Mammary Gland			
Fibroadenomas	2/46	4/45	4/48
Adenocarcinomas	1/46	0/45	1/48
Parathyroid Gland			
Adenomas	0/36	2/41	0/40
Adrenal Gland			
Pheochromocytoma	0/49	1/50	3/50
Carcinomas	1/49	0/50	0/50
Adenomas	0/49	1/50	1/50

[a]Number of lesions/number of animals examined.
[b]Statistically different from control value at $p \leq 0.01$.

Female rats exposed to either Shale or Petroleum JP-5 failed to demonstrate any significant kidney damage. The reason for the sex specificity of the renal lesion remains undetermined.

Lesions in the liver consisted primarily of foci or areas of cellular alteration or hyperplasia. These changes may have been slightly more prevalent in the rats exposed to Petroleum JP-5.

Neither material produced remarkable increases in tumor incidence in the tissues examined. Of minor significance may be the overall pattern of increased endocrine system tumors in rats exposed to Shale or Petroleum JP-5. Although these types of tumors are common to aged rats and may be caused by senile endocrine dysfunction, the relationship to JP-5 exposure should be considered.

A comprehensive technical report on the comparative subchronic toxicity of JP-5 from conventional Petroleum and Shale oil sources will be prepared in the next few months.

THE EVALUATION OF THE CHRONIC TOXICITY OF OTTO FUEL II

Otto Fuel II is used by the Navy as a liquid propellant in weapons systems. The major constituent (≈75%) of Otto Fuel II is 1,2-propylene glycol dinitrate (PGDN), a nitrate ester that produces vasodilation, headache, nasal congestion, nausea and methemoglobinemia upon skin contact or vapor inhalation exposure. PGDN is closely related in structure to trinitroglycerin, which has recently been reported to cause hepatocellular carcinomas in rats (Dacre et al., 1979). This report as well as a lack of toxicity data concerning chronic inhalation of PGDN vapors prompted additional toxicology investigations.

During the period of September 1980 through September 1981 the THRU conducted inhalation exposures to Otto Fuel II using laboratory animals. A complete description of the protocol design was presented in the 1981 Annual Report (MacEwen and Vernot, 1981). Exposures were conducted on an industrial type schedule (6 hours/day, 5 days/week). Two concentration levels, based on analyzed PGDN amounts, were utilized. The low level of 1.4 mg/m^3 PGDN was the existing recommended ceiling threshold limit value. The ACGIH has since reduced the recommended value to 0.3 mg/m^3. The selection of the high level of 240 mg/m^3 PGDN was based on a report by Jones et al. (1972) on 90 day continuous exposure to 236 mg/m^3 PGDN. Species selected for testing included dogs, rats, and mice at the 1.4 mg/m^3 concentration and rats and mice at the 240 mg/m^3 concentration.

Shortly into the exposure period, analysis of dog blood hematologic parameters indicated the presence of anemia. Red blood cell

counts, hemoglobin and hematocrit values of exposed dogs were consistently lower than unexposed control dog values through the course of the exposure. Further indication of a toxic effect was the presence of mild methemoglobinemia in rats exposed to 240 mg/m^3 and in dogs exposed to 1.4 mg/m^3.

At the conclusion of the year long exposure, 10 rats and mice of each sex were killed for complete necropsy and tissue examination. Dogs were also scheduled for examination at that time, but because of the hematologic abnormalities, an additional 60 days of exposure was conducted and then the dogs were killed and examined. Results available through the exposure period, including blood and organ weight analysis, were presented in the 1982 Annual Report (MacEwen and Vernot 1982). Rodents not sacrificed at exposure termination were held for a one year postexposure observation period to determine if any delayed toxic response would occur. The postexposure period ended in September 1982 with the sacrifice and necropsy of all remaining rats and mice.

Exposure to Otto Fuel II vapors did not adversely affect the longevity of mice or rats (Table 14). In fact, groups exposed to Otto Fuel II tended to have lower mortality rates than respective unexposed control groups.

TABLE 14. EFFECTS OF OTTO FUEL II EXPOSURE ON RODENT MORTALITY (%)

	Control N = 100[a]	1.4 mg/m^3 N = 75[a]	240 mg/m^3 N = 100[a]
C57BL/6 Mice, Male	49	25	29
C57BL/6 Mice, Female	64	55	52
Fischer 344 Rats, Male	22	20	21
Fischer 344 Rats, Female	38	27	18

[a]Original number per group. Percentages were calculated after deducting missing animals and accidental deaths.

Male rats exposed to Otto Fuel II vapors had retarded weight gains compared to unexposed male rats (Figure 6). The differences between both test groups and the control group were significant ($p<0.05$) at the majority of the weighing periods. A dose response was indicated by the weight curve. All groups exhibited gradual weight loss toward the conclusion of the study, a common occurrence in aging Fischer 344 rats. Weight differences between the exposure

groups and unexposed control groups became less significant during that period. During the course of the 12 month exposure, body weight means of female rat groups exposed to Otto Fuel II vapors were equal to or greater than unexposed control values (Figure 7). This trend was reversed postexposure when the body weight mean of the 240 mg/m^3 exposure group became significantly ($p<0.01$) less than the unexposed control mean. There was no consistent significant difference in group mean body weights of the 1.4 mg/m^3 exposure group and the unexposed controls postexposure. All group mean weights were essentially equal at the conclusion of the study.

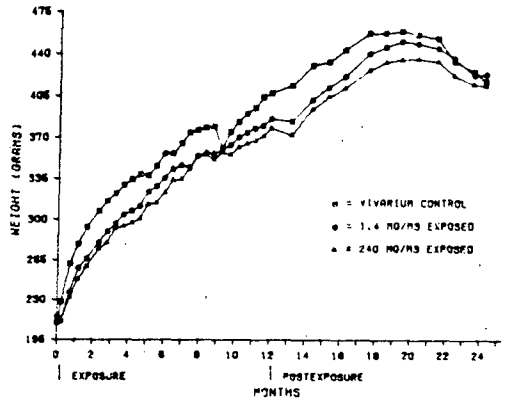


Figure 6. Effect of Otto Fuel II exposure on male rat body weight.

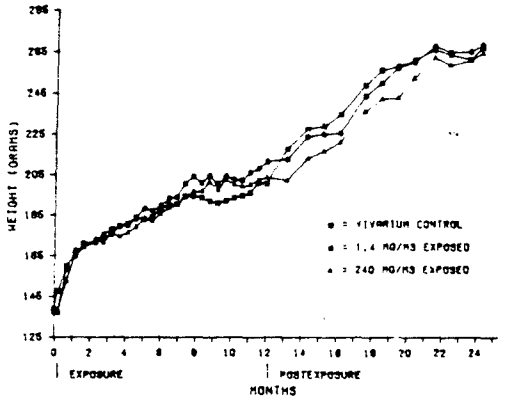


Figure 7. Effect of Otto Fuel II exposure on female rat body weight.

Organ weight measurements obtained at the study conclusion did not indicate any substantial difference between exposed and unexposed rats (Table 15). Organ weight measurements obtained at exposure termination had indicated a slight but statistically significant decrease in liver weights in female rats exposed to Otto Fuel II vapors.

TABLE 15. ORGAN WEIGHTS[a] OF FISCHER 344 RATS ONE YEAR AFTER EXPOSURE TO OTTO FUEL II

MALE

	Control[b]	1.4 mg/m^3	240 mg/m^3
Body Wt, g.	391 ± 8	424 ± 12[c]	394 ± 6
Liver Wt, g.	11.52 ± 0.27	11.38 ± 0.50	11.14 ± 0.54
Liver/100 g. body wt.	2.95 ± 0.05	2.69 ± 0.09	2.83 ± 0.13
Spleen Wt, g.	0.96 ± 0.07	1.18 ± 0.09	1.08 ± 0.16
Spleen/100 g. body wt.	0.25 ± 0.02	0.28 ± 0.02	0.27 ± 0.04
Kidney Wt, g.	3.00 ± 0.11	2.90 ± 0.07	2.79 ± 0.07
Kidney/100 g. body wt.	0.77 ± 0.03	0.69 ± 0.01[c]	0.71 ± 0.01

FEMALE

	Control	1.4 mg/m^3	240 mg/m^3
Body Wt, g.	254 ± 6	249 ± 13	252 ± 7
Liver Wt, g.	6.94 ± 0.21	6.90 ± 0.30	6.62 ± 0.21
Liver/100 g. body wt.	2.74 ± 0.07	2.79 ± 0.06	2.64 ± 0.09
Spleen Wt, g.	0.93 ± 0.15	0.62 ± 0.09	0.55 ± 0.08[c]
Spleen/100 g. body wt.	0.37 ± 0.07	0.25 ± 0.03	0.22 ± 0.04
Kidney Wt, g.	1.79 ± 0.04	1.79 ± 0.05	1.74 ± 0.04
Kidney/100 g. body wt.	0.71 ± 0.01	0.74 ± 0.04	0.69 ± 0.01

[a]Mean ± S.E., N = 10.
[b]Mean ± S.E., N = 9.
[c]Statistically different from controls at $p \leq 0.05$.

Hematology and clinical chemistry values of rats obtained at one-year postexposure are shown in Tables 16 and 17 for male and female rats, respectively. Differences between some exposed and control rat blood parameters are indicated. These are interpreted as incidental findings with no substantial indication of an exposure related effect.

TABLE 16. HEMATOLOGY AND CLINICAL CHEMISTRY VALUES[a] OF MALE RATS ONE YEAR AFTER EXPOSURE TO OTTO FUEL II

	Control	N	1.4 mg/m^3	N	240 mg/m^3	N
RBC (10^6 cells/mm^3)	8.51 ± 0.41	10	8.60 ± 0.47	10	8.36 ± 0.33	9
WBC (10^3 cells/mm^3)	5.1 ± 0.28	10	5.6 ± 0.1	10	5.2 ± 0.2	9
HCT (%)	45 ± 2	10	44 ± 2	10	44 ± 1	9
HGB (g/dl)	17.2 ± 0.6	10	17.3 ± 0.8	10	16.8 ± 0.7	9
MCV (μm^3)	52.7 ± 0.4	10	51.4 ± 0.3[b]	10	52.2 ± 0.6	9
MCH (pg)	20.3 ± 0.3	10	20.2 ± 0.3	10	20.1 ± 0.3	9
MCHC (g/dl)	38.5 ± 0.4	10	39.3 ± 0.4	10	38.6 ± 0.5	9
Total Pro (g/dl)	7.14 ± 0.15	9	7.01 ± 0.1	10	7.06 ± 0.09	10
Albumin (g/dl)	3.70 ± 0.07	9	3.75 ± 0.03	10	3.68 ± 0.05	10
Globulin (g/dl)	3.44 ± 0.11	9	3.32 ± 0.1	10	3.38 ± 0.05	10
A/G Ratio	1.08 ± 0.03	9	1.14 ± 0.03	10	1.09 ± 0.02	10
Glucose (mg/dl)	126 ± 6	10	148 ± 6[b]	10	134 ± 6	10
Total Bilirubin (mg/dl)	0.37 ± 0.02	10	0.34 ± 0.01	10	0.27 ± 0.01[c]	10
Creatinine (mg/dl)	0.5 ± 0.03	10	0.4 ± 0.03	10	0.4 ± 0.03	10
SGPT (IU/L)	39 ± 3	10	33 ± 2[b]	10	33 ± 1[b]	10
SGOT (IU/L)	94 ± 7	10	84 ± 4	10	77 ± 4[b]	10
Alk Phos (IU/L)	5.8 ± 0.4	9	6.3 ± 0.5	10	5.8 ± 0.4	10
BUN (mg/dl)	16.3 ± 1.1	10	16.9 ± 0.5	10	19.2 ± 1.0[b]	10

[a]Mean ± S.E.
[b]Statistically different from control values at $p \leq 0.05$.
[c]Statistically different from control values at $p \leq 0.01$.

Over 1100 animals were included in this study. It is expected that microscopic examination of tissues will be a lengthy procedure; therefore results are not anticipated for some time. The results of the examinations will be included in future annual reports.

TABLE 17. HEMATOLOGY AND CLINICAL CHEMISTRY VALUES[a] OF FEMALE RATS ONE YEAR AFTER EXPOSURE TO OTTO FUEL II

	Control	1.4 mg/m^3	240 mg/m^3
RBC (10^6 cells/mm^3)	7.14 ± 0.22	7.39 ± 0.22	7.30 ± 0.13
WBC (10^3 cells/mm^3)	9.4 ± 3.4	4.6 ± 0.4	4.6 ± 0.4
HCT (%)	39 ± 1	41 ± 1	40 ± 1
HGB (g/dl)	14.5 ± 0.5	15.0 ± 0.3	14.8 ± 0.2
MCV (μm^3)	54.5 ± 0.5	54.9 ± 0.6	55.2 ± 0.4
MCH (pg)	20.3 ± 0.3	20.4 ± 0.3	20.3 ± 0.2
MCHC (g/dl)	37.3 ± 0.6	37.2 ± 0.7	36.8 ± 0.4
Total Pro (g/dl)	7.72 ± 0.09	8.11 ± 0.19	7.80 ± 0.12
Albumin (g/dl)	4.19 ± 0.05	4.41 ± 0.08	4.17 ± 0.09
Globulin (g/dl)	3.53 ± 0.07	3.69 ± 0.12	3.63 ± 0.05
A/G Ratio	1.19 ± 0.03	1.19 ± 0.03	1.15 ± 0.02
Glucose (mg/dl)	137 ± 5	131 ± 9	148 ± 4
Total Bilirubin (mg/dl)	0.24 ± 0.01	0.30 ± 0.02[b]	0.29 ± 0.02[c]
Creatinine (mg/dl)	0.4 ± 0.03	0.4 ± 0.03[d]	0.4 ± 0.03
SGPT (IU/L)	48 ± 3	59 ± 8	49 ± 2
SGOT (IU/L)	91 ± 4	120 ± 26	81 ± 3
Alk Phos (IU/L)	12.9 ± 4.2	6.3 ± 0.6	6.3 ± 0.3
BUN (mg/dl)	19.4 ± 0.6	17.0 ± 0.7[c]	17.8 ± 0.7

[a]Mean ± S.E., N = 10
[b]Statistically different from control values at $p \leq 0.01$.
[c]Statistically different from control values at $p \leq 0.05$.
[d]N = 9

A SUBCHRONIC TOXICITY STUDY OF 90 DAY CONTINUOUS INHALATION EXPOSURE TO PETROLEUM DIESEL FUEL MARINE

Diesel Fuel Marine (DFM) is the standard fuel used by a large number of the ships in the U. S. Naval fleet. Because of the conditions on board a ship where DFM would be stored and used in confined spaces, and because personnel might be exposed for the entire length of a cruise, a continuous exposure for 90 days was chosen for this study.

Diesel Fuel Marine is derived from traditional petroleum sources and is typically a mixture of branched and cyclic hydrocarbons. The fuel contains a small amount of benzene which was considered to be a constituent of major toxicological interest. The

reported effects of benzene exposure involve blood disorders with reductions in the number of erythrocytes, leukocytes and platelets being found in humans after long-term exposure to benzene at high concentrations (Greenberg, 1939; Hardy and Elkins, 1948; Aksoy et al., 1972).

Preliminary to the subchronic exposure study, saturated vapor exposures were conducted. Five male rats were exposed to air saturated with DFM vapors (approximately 2600 mg/m^3) for 8 hours/day for 5 days. There were no deaths nor were there any overt signs of toxicity in animals.

Groups consisting of 6 beagle dogs (3 male, 3 female), 150 Fischer 344 rats (75 male, 75 female), and 140 C57Bl/6 female mice were continuously exposed to concentrations of 50 mg/m^3 or 300 mg/m^3 DFM for 90 days in inhalation chambers. Another group consisting of similar numbers of animals was housed in a separate facility and served as controls. The chambers were maintained at a slightly reduced operating pressure to prevent the escape of contaminant into the work area.

The DFM used in this study was supplied to the Toxic Hazards Research Unit by the U. S. Navy. The material was received from a major petroleum company in 55 gallon steel containers which were tested by quality control procedures to determine uniformity.

DFM vapors were introduced into the domes by passing liquid DFM through a flowmeter to a heated glass evaporator column. The air passing through the evaporator then carried the DFM vapors in the main air supply for the exposure chamber. Concentration of DFM was continuously analyzed by pumping air samples from each chamber into a total hydrocarbon analyzer calibrated with known concentrations of propane. At the conclusion of the DFM exposures, all dogs and 1/3 of the rodents were killed for complete gross evaluation of toxic effects and histopathologic examination. The results of these examinations were described in a previous report (MacEwen and Vernot, 1979). All rodents not killed at the conclusion of the 90 day exposure were held for long-term observation. An interim sacrifice of 1/2 of the remaining rodents occurred 19 months postexposure. The study was terminated in December 1979 and the remaining animals were given complete examinations and tissue specimens were prepared for histopathologic evaluation.

Results of organ weighings and blood analysis obtained at the 19-month interim sacrifice were presented in the 1980 Annual Report (MacEwen and Vernot, 1980).

Since the 1982 Annual Report, pathology examination and evaluation of the rat tissue collected during postexposure observation has been completed.

Non-neoplastic lesions that occurred with some frequency are shown in Table 18. This list has been abbreviated to exclude the lesions of very low incidence. In male rats, the major target organ was the kidney where several lesions demonstrated very clear exposure and dose relationships. Virtually all of the male rats had lesions consistent with progressive renal nephropathy (PRN), common in older rats. However, there was a dose dependent shift in both distribution and severity of the lesions with controls and low dose males generally showing multifocal areas of mild to moderate nephropathy and high dose males exhibiting diffuse moderate to severe nephropathy. There was a striking difference between the male controls and the 300 mg/m^3 DFM exposed males in the occurrence of tubular mineralization and epithelial hyperplasia of the renal papilla. There was also a positive association between the amount of mineralization and epithelial hyperplasia. The hyperplasia generally had a multifocal to segmental distribution and was minimal to mild in severity. All female rat groups, including controls, had a moderately high frequency of PRN. However, no dose response was indicated in the occurrence of this lesion in females and the severity was judged to be minimal to mild in most cases. Reproductive tract cysts were noted with slightly greater frequency in the high dose females when compared to controls.

Neoplastic changes seen in male and female rats are shown in Tables 19 and 20, respectively. The tumors noted in the rats were for the most part common to the species. Statistical analysis of the data failed to indicate any significant ($p<0.05$) increase in tumor formation in DFM exposed rats when compared to controls. Rats exposed to 300 mg/m^3 developed a slightly greater number of tumors overall when compared to controls, however.

Discussion

The major toxic effect of exposure to DFM vapors has been on the rat kidney. Interestingly, injury has been exclusive to male rats. Exposure related renal change has not been found in female rats, female mice or dogs of either sex. Immediately following 90 days of continuous exposure to DFM vapors, hyaline degenerative changes of the cytoplasm of the convoluted renal tubules was seen in 68% of the male rats exposed to 50 mg/m^3 DFM and in 84% of the male rats exposed to 300 mg/m^3 (MacEwen and Vernot, 1979). Male control

rats were free of these changes. An associated lesion (nephropathy) was seen in 96% of the male rats exposed to 300 mg/m^3 DFM, while the control and 50 mg/m^3 DFM exposed male rats were free of this change. The nephropathy consisted of granular casts within the viable collecting tubules and was localized at the corticomedullary junction of the kidney. The casts were considered to be cell debris resulting from epithelial cell necrotic changes in the convoluted segment of the affected nephron.

TABLE 18. SELECTED NON-NEOPLASTIC CHANGES[a] SEEN IN RATS HELD FOR POSTEXPOSURE OBSERVATION AFTER 90 DAY CONTINUOUS INHALATION EXPOSURE TO PETROLEUM DIESEL FUEL MARINE

	Control	50 mg/m^3	300 mg/m^3
Circulatory	-Male-		
Heart fibrosis	32/50	33/48	32/50
Pulmon. Art. Mineralisation	5/50	0/50	9/50
Liver			
Inflammation	5/48	1/48	0/50
Fatty Change	2/48	0/48	1/50
Cellular Change	28/48	30/48	30/50
Bile Duct-Hyperplasia	44/48	44/48	48/50
Kidney			
Nephropathy	50/50	48/50	48/50
Nephrosis	0/50	2/48	2/50
Mineralization	5/50	2/48	43/50[b]
Papillary Hyperplasia	7/50	2/48	18/50[b]
Tubule Degeneration	0/50	0/48	1/50
Testes			
Atrophy	24/48	18/47	29/50
	-Female-		
Lung			
Perivascular Cuffing	24/48	10/50[b]	14/48[b]
Liver			
Inflammation	14/50	13/48	12/48
Fatty Change	7/50	8/48	4/48
Focal Cellular Change	18/50	33/48	22/48
Bile Duct Hyperplasia	14/50	12/48	12/48
Kidney			
Nephropathy	38/48	29/50	29/48
Mineralisation	9/48	3/50	2/48[c]
Papillary Hyperplasia	0/48	0/50	1/48
Reproduction			
Cyst	4/48	11/48	14/48[c]
Endometrial Hyperplasia	10/48	3/48[c]	3/48[c]
Fibroids	11/48	1/48[b]	2/48[b]
Mammary Gland			
Hyperplasia/Adenoma	38/48	34/48	38/48

[a] Number of lesions observed/number of animals examined.
[b] Statistically different from controls at P<0.01
[c] Statistically different from controls at P<0.05.

TABLE 19. NEOPLASTIC CHANGES[a] SEEN IN MALE RATS HELD FOR POSTEXPOSURE OBSERVATION AFTER 90 DAY CONTINUOUS INHALATION EXPOSURE TO PETROLEUM DIESEL FUEL MARINE

	Control	50 mg/m³	300 mg/m³
Skin Subcutaneous			
Benign Tumors	2/48	3/48	4/48
Malignant Tumors	2/48	0/48	0/48
Mammary Gland			
Fibroadenoma	1/36	0/35	0/35
Lung			
Adenocarcinoma	0/50	0/50	1/50
Adenoma	1/50	2/50	1/50
Carcinoma	1/50	0/50	0/50
Nasal Polyp	0/50	1/48	0/49
Liver			
Neoplastic Nodule	2/49	1/49	0/50
Carcinoma	0/49	1/49	2/50
Pancreas Adenoma	0/47	1/48	1/48
Intestine			
Lipoma	1/46	0/48	0/48
Carcinoma	0/46	1/48	0/48
Urinary			
Adenoma (kidney)	0/49	1/48	0/50
Carcinoma (ureter)	0/50	0/50	1/50
Reproductive			
Interstitial Cell Tumor	45/48	39/37	48/50
Mesothelioma	1/49	2/47	1/50
Glands			
Preputial-Adenoma	1/50	2/50	2/50
Pituitary-Adenoma	19/48	13/48	14/47
Adrenal-Neoplasm			
Pheochromocytoma	4/50	4/50	7/48
Thyroid-Adenoma	1/47	3/48	2/38
-Carcinoma	0/47	0/48	1/44
Zymbals-Neoplasm	1/50	0/50	2/50
-Adenoma	0/50	0/50	1/50
Adipose Tissue			
Lipoma	1/50	0/50	0/50
Multiple Organs			
Neoplasm	0/50	0/50	1/50
Sarcoma	1/50	0/50	1/50
Lymphoma	0/50	2/50	1/50
Leukemia	1/50	4/50	7/50
Adenocarcinoma	0/50	0/50	1/50
Total Tumors	87	80	97
Number of Animals with Tumors	46	44	50

[a] Number of lesions observed/number of animals examined.

It is highly probable that the mineral deposition in the renal papillae seen in the male rats exposed to 300 mg/m^3 and held for long term postexposure observation was the direct result of the necrosis occurring in the proximal convoluted tubular epithelium. Mineral deposits were seldom observed in the distal convoluted tubules, collecting ducts, or the urinary space between the wall of the pelvis and the opposing surface of renal papilla. Because of this, it is not probable that the hyperplasia results from the abrasive action of mineralized grit lodged between the epithelial surfaces of the renal papilla and pelvis.

TABLE 20. NEOPLASTIC CHANGES[a] SEEN IN FEMALE RATS HELD FOR POSTEXPOSURE OBSERVATION AFTER 90 DAY CONTINUOUS INHALATION EXPOSURE TO PETROLEUM DIESEL FUEL MARINE

	Control	50 mg/m³	300 mg/m³
Lung			
Adenoma	1/49	0/50	0/49
Liver			
Neoplastic Nodule	1/50	0/49	1/48
Pancreas			
Adenoma	0/48	1/48	1/48
Urinary			
Liposarcoma/Lipoma	2/48	0/50	0/48
Adenoma	1/48	0/50	0/48
Carcinoma (Ureter)	0/50	0/50	1/49
Reproductive			
Adenoma-Clitoral Gland	0/50	1/50	0/50
Carcinoma-Uterus	2/48	1/48	5/49
Leiomyosarcoma	0/48	0/48	1/48
Endometrial Stromal Polyp	8/48	14/48	11/49
Granulosa Cell Tumor	1/48	0/47	0/47
Mammary Gland			
Adenocarcinoma	1/45	0/43	0/43
Fibroadenoma	3/45	2/43	8/43
Adrenal			
Carcinoma	1/50	0/50	1/50
Pheochromocytoma	2/50	1/50	3/50
Thyroid			
Follicular Cell Adenoma	0/41	0/46	1/46
C-Cell Adenoma	0/41	1/46	1/46
Carcinoma	1/41	0/46	0/46
Adenoma (Parathyroid)	1/34	0/33	0/36
Multiple Organs			
Neoplasm	1/50	0/50	0/49
Lymphoma	1/50	0/50	0/49
Leukemia	5/50	5/50	8/49
Sarcoma	0/50	0/50	1/49
Pituitary			
Carcinoma	0/49	0/45	2/48
Adenoma	29/49	24/45	25/48
Total Tumors	64	50	70
Number of Animals with Tumors	43	34	37

[a] Number of lesions observed/number of animals examined.

A more likely hypothesis is that the presence of appreciable amounts of impacted mineral present in the loops of Henle serves to induce an undesirable rigidity to the papillae which may enhance a "friction-rub" action between the epithelial surfaces of the renal papilla and pelvis.

Renal tubular necrosis is probably the end product of severe hyaline droplet degeneration. It is likely that much of the hyaline droplet change observed in male rats at exposure termination was reversible since it is a normal physiological component of the resorption process of the tubule epithelial lining. Most likely, it is when the protein clearing process is overloaded that necrosis

ensues. The effect of hydrocarbon burden, in this case DFM, on this process is clearly evident; however, the exact mechanism is as yet undetermined.

Under the conditions of this study, petroleum DFM exhibited no carcinogenic activity. The results indicate no substantial increase in tumor formation in DFM exposed rats when compared to controls. Tumors noted in all groups were common to the species. Evidence was presented in the 1982 annual report supporting a similar conclusion regarding female mice (MacEwen and Vernot, 1981).

A SUBCHRONIC TOXICITY STUDY OF 90 DAY CONTINUOUS INHALATION EXPOSURE TO SHALE DIESEL FUEL MARINE

This study was conducted as a companion study to a previous subchronic inhalation study of Diesel Fuel Marine (DFM) derived from petroleum sources. The protocol, chemical generation, analytical systems, clinical chemistry, body and organ weight results are described in the 1980 THRU Annual Report (MacEwen & Vernot, 1980). The basic design of the study was patterned after other 90 day studies conducted by the THRU and involved tissue examination of a portion of the animal groups at exposure termination as well as long term holding for determination of postexposure effects. At the conclusion of the 90 day exposure period, indications of toxicity seen in male rats exposed to 300 mg/m^3 Shale DFM included increased BUN and creatinine levels, and increased kidney and liver weight.

Histopathology examination of the tissues collected from the animals at exposure termination has been completed. Test groups included 3 male and 3 female beagle dogs. A number of common tissue lesions were observed in the dogs exposed to 50 or 300 mg/m^3 Shale DFM. Frequency of occurrence and severity of the noted lesions were not increased in Shale DFM exposed groups compared to the control group. Exposure to Shale DFM vapors at concentrations up to 300 mg/m^3 had no measurable effect on beagle dogs.

Lesions in mice considered to be associated with exposure to Shale DFM were limited to the lung and liver as shown in Table 21.

TABLE 21. NON-NEOPLASTIC LESIONS[a] IN FEMALE MICE IMMEDIATELY FOLLOWING CONTINUOUS EXPOSURE TO SHALE DFM VAPORS FOR 90 DAYS

	Control	50 mg/m^3	300 mg/m^3
Lung			
Perivasculitis	12/49	34/49[b]	38/48[b]
Liver			
Fatty Change (vacuolization)	18/51	46/49[b]	41/47[b]

[a]Number of lesions observed/number of animals examined.
[b]Statistically different from control value, $p \leq 0.01$.

Pulmonary inflammatory changes consisting predominantly of mild peribronchiolar and perivascular mononuclear infiltrates were observed in 69% and 79% of the mice assigned to the low and high dose groups, respectively. In contrast, only 24% of the control mice exhibited significant pulmonary inflammatory infiltrates. Although infectious agents could not be completely ruled out as etiologic factors, the marked increase in inflammatory changes in exposed subjects strongly suggests a relationship with Shale DFM exposure.

Hepatocellular vacuolization was the only significant exposure-associated alteration in the liver. This cytoplasmic vacuolar change was consistent with fatty metamorphosis. However, special staining techniques needed to specifically identify cytoplasmic fat were not done. Several patterns of vacuolar change, based on hepatic lobular distribution, were present. Approximately 35% of the control mice had diffuse, minimal tc mild centrilobular cytoplasmic vacuolization, whereas 94% of the low dose and 87% of the high dose subjects manifested panlobular cytoplasmic vacuolar change. Furthermore, cytoplasmic vacuolization in exposed mice was graded as mild to moderate in severity, as compared with only minimal to mild changes in unexposed controls.

Lesions in rats considered to be associated with exposure to Shale DFM were limited to the kidneys of male test animals as shown in Table 22.

Dose dependent renal lesions present only in male treatment groups included hyaline degeneration and necrosis of what was believed to be predominantly proximal convoluted tubules.

TABLE 22. NON-NEOPLASTIC LESIONS[a] IN THE RENAL CORTEX OF MALE RATS IMMEDIATELY FOLLOWING CONTINUOUS EXPOSURE TO SHALE DFM VAPORS FOR 90 DAYS

	Control	50 mg/m^3	300 mg/m^3
Interstitial inflammation	4/25	4/24	20/25[b]
Hyaline degeneration	0/25	23/24[b]	25/25[b]
Necrosis	0/25	0/24	25/25[b]

[a]Number of lesions observed/number of animals examined.
[b]Statistically different from the control value at $p < 0.01$.

Chronic inflammation of the renal cortical interstitial tissue was also observed with increased frequency in the high dose male rats and was often associated with degenerative tubular lesions. This inflammatory process was characterized by focal to multifocal, minimal to mild aggregations of lymphocytes, plasma cells, and macrophages, and occasionally was accompanied by the early deposition of fibrous connective tissue. These cortical interstitial changes could not be distinguished from early manifestations of progressive nephropathy which is common in older rats, especially males. However, the early onset of these interstitial lesions and their association with tubular degeneration and necrosis in this experiment strongly suggests that they are an extension of the nephropathologic changes in male rats exposed to Shale DFM.

Renal tubular hyaline degeneration was observed in virtually 100 percent of both low and high dose groups. The severity of the degeneration ranged from minimal to mild and mild to moderate in the low and high dose animals, respectively.

Necrosis of renal tubular epithelial cells was evident in multifocal regions of the outer and middle cortex in 100 percent of the high dose group, and it was considered to be an irreversible variant of the hyaline degenerative process. Necrosis was not seen as a distinctive feature of the low dose animals.

The types of tissue lesions seen in animals exposed to Shale DFM are very similar to those found in animals exposed to Petroleum DFM (MacEwen & Vernot, 1979). The principal sites of toxicity resulting from exposure to Petroleum or Shale DFM were the liver and kidney. Mild respiratory irritation seen in rats exposed to Petroleum DFM and mice exposed to Shale DFM was similar in etiology to chronic respiratory disease. Fatty liver change was seen in Shale DFM exposed mice while liver inflammation was seen in Petroleum DFM

exposed mice. By far the most striking and consistent effect noted after inhalation of DFM vapors was the nephrotoxicity in male rats. A clear dose response in microscopic tissue alterations was seen with either Petroleum DFM or Shale DFM - hyaline degeneration at either exposure level accompanied by epithelial cell necrosis at the high level.

Examination of tissues obtained from the rats held for observation after exposure to Petroleum DFM indicated the renal necrosis had progressed to mineralization and hyperplasia of the renal papilla. Examination of the tissues from Shale DFM exposed rats held for long term observation is still in progress and it has not been determined to what degree renal damage has progressed.

EVALUATION OF THE CHRONIC TOXICITY OF JP-7 JET FUEL

As part of a continuing program of evaluation of the toxicity of hydrocarbon fuels used by the Air Force, the THRU conducted an inhalation exposure study using the jet fuel JP-7. JP-7 is a complex mixture of aliphatic and aromatic hydrocarbons which closely resembles the U. S. Navy jet fuel, JP-5. The study conducted was an industrial type involving exposure of Fischer 344 rats and C57BL/6 mice to JP-7 vapors for 6 hours/day, 5 days/week. Exposures were not conducted on weekends or holidays. The exposure phase of the study ran from April 1981 to April 1982. A small sample of the animals was taken for evaluation of chronic toxicity response during exposures. The remaining animals were held for one year postexposure observation.

Two chambers were utilized for the exposures; one contained a concentration of 150 mg/m^3 and the other contained a concentration of 750 mg/m^3. Animal groups consisted of 100 male and 100 female Fischer 344 rats and C57BL/6 mice, 9-11 weeks of age at exposure initiation, obtained from Charles River Breeding Laboratories. An additional group with the same numbers of animals was housed in another Thomas Dome Chamber to serve as sham operated controls. All animals had food and water ad libitum during nonexposure hours. Food was removed during the exposure period.

The contaminant introduction and analysis system used for JP-7 vapor was similar to the systems used for other fuel studies. A detailed discussion of the system can be found in a previous annual report discussing an inhalation study of the Jet Fuel JP-4 (MacEwen and Vernot, 1980). The only major modification to that method was

the use of heptane rather than propane as the primary hydrocarbon calibration standard.

Following the 1-year exposure period, 12 of each species and sex from all groups were killed for tissue collection and examination. The remaining rodents were held for one year of postexposure observation. At the conclusion of this period (April, 1983), all remaining rodents were killed for tissue collection and examination.

All animals were observed hourly during the exposure and 4 times daily during the postexposure holding period. More frequent examinations were made toward the end of the study when natural mortality increases and moribund animals were killed to prevent losses of tissue.

Rats were individually weighed at biweekly intervals during exposure and monthly during the postexposure period. Mice were weighed in groups with the group mean weights followed on a monthly basis throughout the experimental period.

All animals that died or were killed were necropsied and tissues collected for histopathologic examination in accordance with the NCI protocol. Electron microscopic examination is also being conducted on a small sample of rats from each group at the scheduled sacrifices.

Since the 1982 annual report, the study was terminated and final gross observations and measurements were made on all remaining animals. Tissues were taken and prepared for histologic examination. This occurred in April and May of 1983.

Body weight curves for the Fischer rats are shown in Figures 8 and 9 for male and females, respectively. Body weights of male rats exposed to JP-7 at 750 mg/m^3 were generally less than sham exposed controls. The difference was often statistically significant at $p < 0.05$. Exposure to JP-7 at 150 mg/m^3 had no sustained effect on male rat body weight. Both groups of female rats exposed to JP-7 were consistently heavier than sham exposed controls. No dose response was indicated.

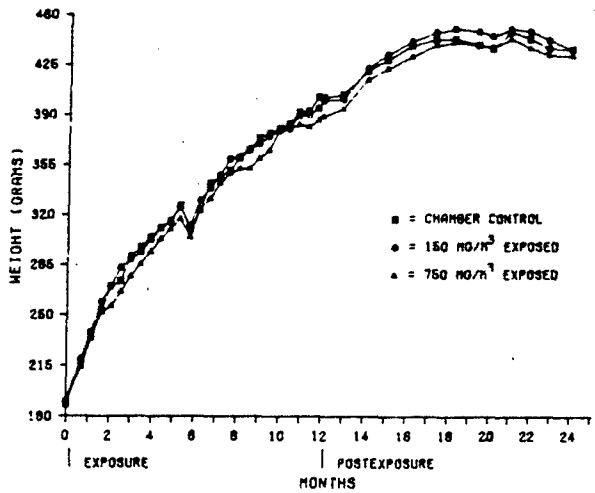


Figure 8. Effect of inhalation exposure to JP-7 on male rat body weight.

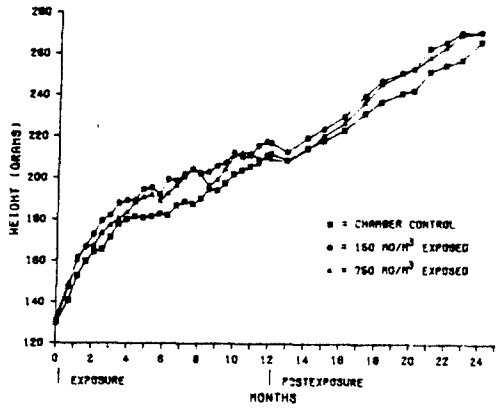


Figure 9. Effect of inhalation exposure to JP-7 on female rat body weight.

Mortality ratios shown in Table 23 indicate that exposure to JP-7 vapors did not adversely affect the life span of the rodents.

Results of histopathology examination of the tissues collected during the study will be discussed in future annual reports.

TABLE 23. MORTALITY IN ANIMALS EXPOSED TO JP-7 AT TERMINATION OF THE TWO YEAR STUDY[a]

	Chamber Control	150 mg/m³	750 mg/m³
C57BL/6 Mice, Female	53/87	71/88	64/88
C57BL/6 Mice, Male	57/87	40/88	50/87
Fischer 344 Rats, Male	28/88	30/88	30/88
Fischer 344 Rats, Female	24/88	15/88	24/88

[a]Originally 100 animals/group, 12/group were killed at exposure termination. Missing and accidental deaths have been deleted from the mortality ratios.

THE EXPERIMENTAL DETERMINATION OF THE CHRONIC TOXICITY OF JP-TS JET FUEL

The U. S. Air Force requested a long-term inhalation study to determine the chronic toxicity of the high altitude jet fuel designated JP-TS. This jet fuel is similar in composition to the jet fuel JP-4 previously investigated by the Toxic Hazards Research Unit.

Intermittent exposure for one year to 5000 mg/m³ JP-4 vapor produced organ hypertrophy and bronchial irritation in rats as well as CNS effects and osmotic erythrocyte fragility increases in female dogs. The reason for the organ hypertrophy in rats was not clear but appeared to be of little toxicologic significance since no tissue destruction or alteration was observed. The increase in RBC osmotic fragility appeared to have been a real effect of unknown etiology which was transient in nature. The central nervous system effect seen in dogs and respiratory irritation in rats were effects which could be considered relevant to possible human experience with chronic exposure to JP-4 vapor.

A 90 day continuous exposure of rats, mice, and dogs to 1000 and 500 mg/m³ JP-4 has also been completed in our laboratory. The dogs in this study exhibited no CNS effects nor were any increases of osmotic fragility noted during the course of the study.

This study was designed to determine the chronic effects, including oncogenic, of long-term inhalation exposure of rats and mice to JP-TS jet fuel vapor. The same exposure regimen was followed as

in previous experiments to investigate chronic toxicity. The results of this experiment will be used for comparison with studies done previously on fuels of a similar chemical nature.

JP-TS is a broad mixture of aliphatic and aromatic hydrocarbon compounds defined in terms of physical and chemical characteristics and includes various additives, all of which meet the requirements of Military Specificiation MIL-T-25524B. Pertinent chemical and physical properties of the fuel detailed in the military specifications are listed below:

Sulfur, max.	0.3% (by wt.)
Mercaptan sulfur, max.	0.001% (by wt.)
Aromatics, max.	20.0% (by vol.)
Olefins, max.	3.0% (by vol.)
Distillation:	
Initial boiling point, °F	315
End point, °F	500
Freezing point, °F, max.	-64
Flash point, °F, min.	110
Viscosity, centistokes at -40°F, max.	12.0

Mice and rats were exposed for one year to 200 and 1000 mg/m^3 JP-TS vapor by the inhalation route in Thomas Dome chambers using an industrial work week schedule of 6 hours/day, 5 days/week with holidays and weekends excluded to simulate a human exposure regimen. Each exposure group consisted of 100 male and 100 female Fischer 344 rats and 100 male and 100 female C57BL/6 mice. A sham treated control group was housed in a Thomas Dome chamber. The controls served in the same capacity for studies conducted concurrently with JP-7. The animals were caged according to ILAR standards for laboratory animal care.

Following the exposure period, 12 animals from each group were killed, two for electron microscopic and ten for light microscopy examination. The remaining rodents were held one year for postexposure observation. All animals have now been necropsied and tissues are undergoing histopathologic examination.

A previous annual report (MacEwen and Vernot, 1982) details the experimental protocol for the one-year inhalation exposure of rats and mice to JP-TS fuel and gives results of effects on blood parameters and organ weights of male and female rats killed at exposure termination.

Results

Mean body weights for the rat groups obtained on a biweekly schedule through 12 months of exposure and monthly thereafter are shown in Figures 10 and 11. Both groups of exposed male rats showed a slight depression in mean body weight throughout most of the 24 months. The female control group failed to gain weight at a rate equal to the exposed groups during the first ten months of the study. Thereafter, the female control mean weights paralleled the test groups but remained slightly lower through the end of the study. Mouse mean weights revealed no exposure-related effects.

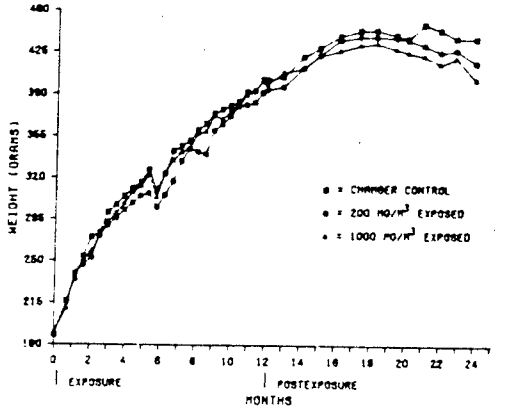


Figure 10. Mean body weight of male rats exposed for one year to JP-TS.

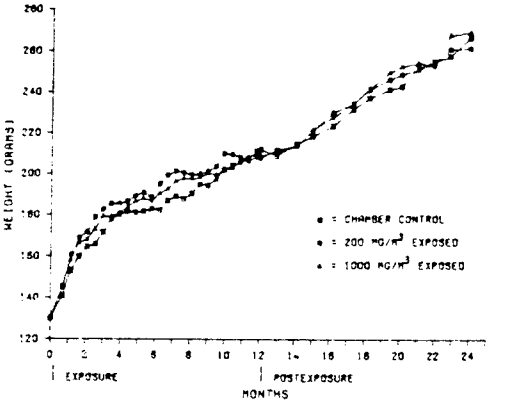


Figure 11. Mean body weight of female rats exposed for one year to JP-TS.

The final mortality ratios of rats and mice at the end of 24 months are shown in Table 24. The numbers of natural deaths or moribund sacrifices that occurred during the study were not above expectations and were independent of exposure concentrations.

TABLE 24. MORTALITY OF ANIMALS EXPOSED TO JP-TS AT TERMINATION OF THE TWO YEAR STUDY[a]

	Chamber Controls	200 mg/m^3	1000 mg/m^3
Mice, Male	57/87	63/86	37/88
Mice, Female	53/87	68/88	56/87
Rats, Male	28/88	26/88	23/87
Rats, Female	24/88	15/88	18/88

[a]Originally 100 animals/group, 12/group sacrificed at exposure conclusion except for 1000 mg/m^3 exposed female mice, where 11/group were sacrificed. Missing animals and accidental deaths have been deleted.

Conclusions concerning the chronic toxicologic effects of inhaled JP-TS on rats and mice await completion of the histopathology examination of tissues and will be discussed in future reports.

A SUBCHRONIC TOXICITY STUDY OF 90 DAY CONTINUOUS INHALATION EXPOSURE TO JP-8 JET FUEL

The THRU has been conducting a series of inhalation studies investigating the health hazards associated with hydrocarbon fuels used by the Air Force. One fuel not previously tested is the jet fuel JP-8.

JP-8 is a complex mixture of aliphatic and aromatic hydrocarbons and is similar to two other kerosene type fuels (JP-4 and JP-5) which have already been tested. Inhalation studies with JP-4 and JP-5 have used 90 day continuous exposure regimens. Two contaminant concentrations have typically been used with the higher concentration being 1000 mg/m^3 and 750 mg/m^3 for JP-4 and JP-5, respectively.

Previous studies of hydrocarbon fuels have shown histopathologic changes in the kidneys of male rats at exposure termination (MacEwen and Vernot, 1978, 1981). Due to the similarity of JP-8,

JP-4, and JP-5, it was considered highly likely that renal damage would also occur in male rats exposed to JP-8. To further evaluate this renal toxicity, routine urinalysis and additional sacrifices for rat tissue examination were included in the JP-8 study design. Sampling in this manner allows for microscopic evaluation of kidney tissue prior to onset of chronic nephropathy common to older rats.

Also included in the study design were pulmonary clearance and pulmonary function tests conducted by the respiratory physiology group.

Methods

JP-8 is a complex mixture of hydrocarbon compounds refined from petroleum that is defined in terms of physical and chemical characteristics and includes various additives, all of which meet the requirements of Military Specification MIL-T-83113. The allowable physical and chemical parameters are detailed below:

Aromatics, vol. % max:	25
Mercaptan Sulfur, wt. %, max:	0.001
Sulfur, total, wt. %, max:	0.4
Olefins, vol%, max:	5
Distillation, °C	
End Point, max. temp:	300
Flash Point, min, °C:	38
Density, kg/m^3, min at 15°C:	775
Density, kg/m^3, max at 15°C:	840
Freezing Point, °C, max:	-50

Mice and rats were exposed to 500 mg/m^3 and 1000 mg/m^3 JP-8 vapor on a continuous basis for 90 days. Two Thomas Dome inhalation chambers were utilized for these exposures. Sham exposed controls were maintained in a separate inhalation chamber. Each chamber housed 95 male and 75 female Fischer 344 rats and 100 male and 100 female C57BL/6 mice.

Following the 90 day continuous exposure period, 15 rats of each sex and 25 mice of each sex from each group were killed for tissue collection and examination. Additional interim samplings were conducted at 2 weeks postexposure (10 male rats/group) 2 months postexposure (10 male rats/group), and 9 months postexposure (10 rats and mice of each sex/group). All remaining animals will be necropsied during the 24th month of the study.

For the purposes of pulmonary testing, an additional 28 male rats were included for exposure to the 1000 mg/m^3 concentration. A similar number was housed in the control chamber.

The introduction system used to generate JP-8 vapors was similar to that used in previous jet fuel inhalation studies. A detailed discussion of this system can be found in the 1982 annual report (MacEwen and Vernot, 1982) in the discussion of JP-7 chronic toxicity. The system basically consisted of heated glass evaporation towers through which JP-8 liquid was passed. An air stream flowing through the towers carried the vapors into the main chamber air supply. Analysis of the chamber concentration was accomplished by pumping air samples from each chamber into a total hydrocarbon analyzer calibrated with heptane.

Gas chromatographic fingerprints were obtained from a liquid sample of each drum of JP-8 prior to the initiation of the study. A GC fingerprint of a sample of each fuel was also obtained as the fuel was introduced into the exposure system to insure constancy of the JP-8 over the course of the exposure. GC fingerprints of the contaminant in the chamber were obtained every two weeks. A Royco® particle counter equipped with a 508 digital monitor was used to measure possible formation of vapor condensate aerosol.

Rats were weighed individually at biweekly intervals during exposure and monthly during the postexposure period. Mice were weighed monthly throughout the study in groups according to the number of animals per cage.

At the completion of the exposure phase, blood was collected for the tests shown in Table 25 from the rats killed for tissue examination.

The tests listed in Table 25 will also be performed on all rats killed at 9-months postexposure and on 10 male and 10 female rats from each group at the completion of the study. Blood for hematology, creatinine and BUN measurements was collected from the 10 male rats killed at 2 weeks and 2 months postexposure.

Urine samples are being collected from male rats for the tests shown in Table 26.

The urine samples were collected once preexposure and will be collected at each scheduled sacrifice period. Rats were placed in individual plastic metabolism cages for a 6 nour urine collection.

TABLE 25. CLINICAL HEMATOLOGY AND CHEMISTRY TESTS PERFORMED ON RATS EXPOSED TO JP-8 JET FUEL

Hematology	Chemistry
Hematocrit	Sodium
Hemoglobin	Potassium
RBC	Calcium
WBC	Albumin/Globulin
Differentials	Total Protein
Mean Corpuscular Volume (MCV)	Glucose
Mean Corpuscular Hemoglobin (MCH)	Alkaline Phosphatase
Mean Corpuscular Hemoglobin Concentration (MCHC)	SGPT
	SGOT
	Bilirubin
	Creatinine
	BUN

TABLE 26. URINALYSIS PERFORMED ON MALE RATS EXPOSED TO JP-8 JET FUEL

pH	Blood
Protein	Nitrate
Glucose	Urobilinogen
Ketone	Osmolality
Bilirubin	Microscopic

At the completion of the 90 day exposure period, 12 randomly selected male rats each from the control group and the 1000 mg/m^3 JP-8 exposed group were placed in plastic metabolism cages for overnight urine collection. This urine will be used for GC/MS analysis of metabolites.

Whole body, liver, kidney and spleen weights of rats were measured at exposure termination. These weights will also be measured in sample rats at 9 months postexposure and at study termination.

All animals that die or are killed during the study are necropsied with tissue collected for histopathologic examination in accordance with NCI protocol. Electron microscopic examination is also being conducted on a small sample of rats and mice from each group of animals at exposure termination.

Results

The body weights of male rats are shown in Figure 12. A dose related depression in body weight gain was seen throughout the exposure period with the difference in body weights between control and both JP-8 exposed groups statistically significant at $p \leq 0.01$. After 12 weeks of exposure, mean body weights of male rats exposed to 1000 mg/m^3 JP-8 were approximately 92% of the unexposed control value. The body weights of female rats are shown in Figure 13. While the body weights of female rats exposed to 1000 mg/m^3 JP-8 were generally significantly less ($p \leq 0.05$) than control rat body weights through the exposure period, the effect was not as prominent as in male rats.

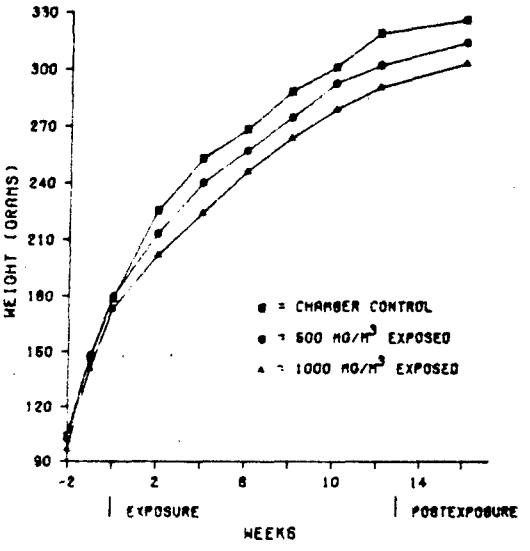


Figure 12. Effect of JP-8 exposure on male rat body weight.

The results of tests conducted on male rat blood collected at exposure termination are shown in Table 27. Low red blood cell counts were noted in male rats exposed to 1000 mg/m^3 JP-8 vapors and also, to a lesser extent, in those exposed to 500 mg/m^3. Evidence of kidney dysfunction was indicated by increased BUN and creatinine values in both exposure groups.

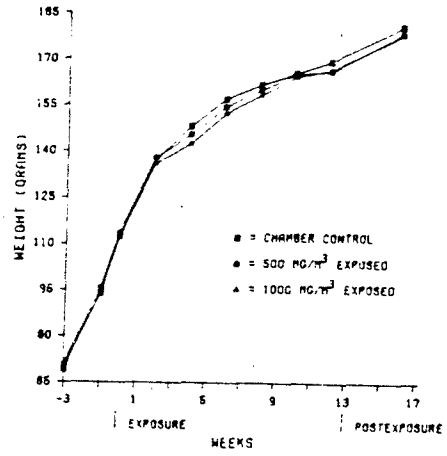


Figure 13. Effect of JP-8 exposure on female rat body weight.

Female rat blood test results are shown in Table 28. Low red cell counts were not a significant finding in female rats. Protein and electrolyte results are not given since they were not done on a significant number of animals. Although the red blood cell counts of the 1000 mg/m^3 exposure group were lower than control values, the difference was slight and not significant at $p \leq 0.05$. There was no indication of kidney dysfunction in the female JP-8 exposed rats.

Blood values of the male rats killed at 2 weeks postexposure are shown in Table 29. JP-8 exposed male rats continued to have slightly low red blood cell values. BUN levels were also elevated in these rats compared to controls. The increased creatinine levels evident in the exposed rats at exposure termination had returned to normal control level by 2 weeks postexposure.

Organ weights measured at the exposure termination sampling revealed increased liver weight in both male and female rats exposed to JP-8 vapors (Table 30). In male rats the increased weight was evident only at the 1000 mg/m^3 exposure level, while increased liver weight was found in both groups of exposed female rats. Exposure to either concentration of JP-8 produced substantial elevations in kidney weights in male rats and a dose response was evident in both absolute and organ ratio values.

TABLE 27. EFFECT OF 90 DAY CONTINUOUS INHALATION OF JP-8 VAPORS ON MALE RAT BLOOD[a]

	Control	N	500 mg/m^3	N	1000 mg/m^3	N
RBC (x10^6 cells/mm^3)	8.48 ± 0.15	12	8.03 ± 0.13[b]	11	7.48 ± 0.10[c]	10
WBC (x10^3 cells/mm^3)	5.8 ± 0.3	12	5.1 ± 0.3	11	7.0 ± 0.3[b]	10
HCT (%)	43.6 ± 0.6	12	41.8 ± 0.8	11	38.3 ± 0.6[c]	10
HGB (g/dl)	15.6 ± 0.3	12	14.7 ± 0.2[b]	11	14.7 ± 0.2[b]	10
MCV (cu microns)	51.4 ± 0.2	12	52.0 ± 0.2[b]	11	51.2 ± 0.2	10
MCH (micro-micrograms)	18.4 ± 0.1	12	18.4 ± 0.1	11	19.7 ± 0.2[c]	10
MCHC (%)	35.8 ± 0.3	12	35.3 ± 0.3	11	38.5 ± 0.4[c]	10
Tot. Protein (g/dl)	6.92 ± 0.08	15	6.92 ± 0.05	15	6.99 ± 0.07	15
Albumin (g/dl)	4.43 ± 0.05	15	4.41 ± 0.05	15	4.54 ± 0.05	15
Globulin (g/dl)	2.49 ± 0.04	15	2.51 ± 0.06	15	2.45 ± 0.07	15
A/G Ratio	1.79 ± 0.03	15	1.79 ± 0.09	15	1.89 ± 0.09	15
Glucose (mg/dl)	161 ± 7	15	144 ± 6	15	140 ± 3[c]	15
Potassium (mEq/L)	6.4 ± 0.3	13	6.4 ± 0.3	12	6.4 ± 0.2	13
Sodium (mEq/L)	167 ± 5	13	168 ± 4	12	165 ± 3	13
Calcium (mg/dl)	0.2 ± 0.1	15	10.7 ± 0.1	15	10.8 ± 0.1	15
Bilirubin (mg/dl)	0.2 ± 0.01	15	0.2 ± 0.01	14	0.2 ± 0.01	15
Creatinine (mg/dl)	0.56 ± 0.02	15	0.66 ± 0.02[c]	14	0.78 ± 0.02[c]	15
SGPT (IU/L)	47 ± 3	15	37 ± 2[b]	15	35 ± 2[c]	15
SGOT (IU/L)	94 ± 3	15	82 ± 3[c]	15	94 ± 2	15
Alk. Phos (IU/L)	9.4 ± 0.4	15	10.0 ± 0.3	15	11.3 ± 0.4[c]	15
BUN (mg/dl)	15.2 ± 0.4	15	18.0 ± 0.4[c]	15	18.7 ± 0.9[c]	15

[a]Mean ± S.E.
[b]Statistically different from control value at $P \leq 0.05$.
[c]Statistically different from control value at $P \leq 0.01$.

TABLE 28. EFFECT OF 90 DAY CONTINUOUS INHALATION OF JP-8 VAPORS ON FEMALE RAT BLOOD[a]

	Control	N	500 mg/m^3	N	1000 mg/m^3	N
RBC (x10^6 cells/mm^3)	7.45 ± 0.15	10	7.30 ± 0.11	14	7.24 ± 0.20	13
WBC (x10^3 cells/mm^3)	4.8 ± 0.3	10	4.3 ± 0.3	14	5.4 ± 0.3	13
HCT (%)	40.0 ± 0.8	10	39.2 ± 0.6	14	39.1 ± 1.0	13
HGB (g/dl)	15.2 ± 0.3	10	15.0 ± 0.2	14	15.2 ± 0.3	13
MCV (cu microns)	53.6 ± 0.2	10	53.7 ± 0.2	14	54.0 ± 0.3	13
MCH (micromicrograms)	20.4 ± 0.2	10	20.5 ± 0.3	14	21.0 ± 0.2	13
MCHC (%)	38.0 ± 0.4	10	38.2 ± 0.6	14	38.8 ± 0.3	13
Glucose (mg/dl)	131 ± 4	8	117 ± 5[b]	9	120 ± 6	7
Calcium (mg/dl)	11.3 ± 0.1	10	11.2 ± 0.2	13	11.0 ± 0.3	10
Bilirubin (mg/dl)	0.2 ± 0.0	3	0.3 ± 0.02	6	0.3 ± 0.3	4
Creatinine (mg/dl)	0.60 ± 0.0	4	0.54 ± 0.05	5	0.63 ± 0.3	4
SGPT (IU/L)	51 ± 4	12	35 ± 2[b]	14	36 ± 1[b]	15
Alk. Phos. (IU/L)	5.4 ± 0.3	12	5.1 ± 0.2	14	7.7 ± 0.8[c]	12
BUN (mg/dl)	16.7 ± 0.4	11	15.6 ± 0.5	13	16.8 ± 0.5	11

[a]Mean ± S.E.
[b]Statistically different from control values at $p \leq 0.01$.
[c]Statistically different from control values at $p \leq 0.05$.

TABLE 29. SELECTED HEMATOLOGY AND SERUM CHEMISTRY VALUES OF MALE RATS TWO WEEKS AFTER EXPOSURE TO JP-8[a] VAPORS

	Control	500 mg/m^3	1000 mg/m^3
RBC (10^6 cells/mm^3)	8.36 ± 0.13	7.59 ± 0.12[b]	7.94 ± 0.09[c]
WBC (10^3 cells/mm^3)	7.8 ± 0.4	6.8 ± 0.3[c]	7.0 ± 0.3
HCT (%)	41.8 ± 0.7	38.5 ± 0.8[b]	42.0 ± 0.6
HGB (g/dl)	16.4 ± 0.2	14.7 ± 0.1	14.5 ± 0.1[b]
BUN (mg/dl)	15.2 ± 0.2	16.4 ± 0.3[c]	17.8 ± 0.4[b]
Creatinine (mg/dl)	0.54 ± 0.02	0.56 ± 0.02	0.56 ± 0.02

[a]Mean ± S.E., N = 10
[b]Statistically different from control value $p \leq 0.01$.
[c]Statistically different from control value $p \leq 0.05$.

TABLE 30. EFFECT OF 90 DAY CONTINUOUS INHALATION OF JP-8 VAPORS ON RAT ORGAN WEIGHT [a]

	Male		
	Control	500 mg/m^3	1000 mg/m^3
Body Weight (gm)	308 ± 5	293 ± 5[b]	283 ± 5[c]
Liver Weight (gm)	8.47 ± 0.21	8.19 ± 0.20	9.22 ± 0.25[b]
Liver/100 gm body wt.	2.74 ± 0.04	2.83 ± 0.04	3.24 ± 0.04[c]
Spleen Weight (gm)	0.59 ± 0.01	0.61 ± 0.01	0.59 ± 0.01
Spleen/100 gm body wt.	0.19 ± 0.002	0.21 ± 0.002[c]	0.21 ± 0.002[c]
Kidney Weight (gm)	2.04 ± 0.03	2.53 ± 0.06[c]	2.91 ± 0.06[c]
Kidney/100 gm body wt.	0.66 ± 0.01	0.87 ± 0.01[c]	1.02 ± 0.02[c]

	Female		
	Control	500 mg/m^3	1000 mg/m^3
Body Weight (gm)	162 ± 2	172 ± 2	157 ± 2[b]
Liver Weight (gm)	4.43 ± 0.09	4.84 ± 0.11[b]	4.88 ± 0.12[c]
Liver/100 gm body wt.	2.74 ± 0.04	2.98 ± 0.05[c]	3.13 ± 0.07[c]
Spleen Weight (gm)	0.40 ± 0.01	0.41 ± 0.01	0.41 ± 0.01
Spleen/100 gm body wt.	0.24 ± 0.01	0.25 ± 0.002	0.26 ± 0.01[b]
Kidney Weight (gm)	1.17 ± 0.02	1.21 ± 0.03	1.21 ± 0.02
Kidney/100 gm body wt.	0.72 ± 0.01	0.75 ± 0.02	0.77 ± 0.01[c]

[a]Mean ± S.E., N = 13 to 15 rats/group
[b]Statistically different from controls at $p \leq 0.05$.
[c]Statistically different from controls at $p \leq 0.01$.

The kidney to body weight ratio of the 500 mg/m^3 exposure group was 32% greater than the control value, while an increase of 52% was seen in the rats exposed to 1000 mg/m^3. The kidney to body weight ratio of the female rats exposed to 1000 mg/m^3 was also greater than the control value; however, the elevation was much less than that of the male rats exposed to this concentration. Increased spleen to body weight ratios were minor and probably incidental to exposure.

The majority of the urinalysis results failed to indicate substantial differences between preexposure examination and postexposure examination but an effect was seen in urine concentrating capability as shown in Table 31. At exposure termination urine osmolality of rats exposed to 1000 mg/m^3 was significantly less ($p \leq 0.05$) than control rat urine osmolality. Interestingly, the control rat urine osmolality at exposure termination was considerably less than the preexposure value, an anomalous effect possibly related to chamber housing. At 2 weeks postexposure the urine osmolality of control rats had returned to the preexposure level. JP-8 exposed animals, while showing recovery from exposure termination, had not yet returned to preexposure levels and in both exposure groups, urine osmolality values were significantly less ($p \leq 0.05$) than the control value. Microscopic examination of the urine collected at exposure termination revealed epithelial cells in the rats exposed to JP-8 (Table 32). These cells were noted in the urine of all ten of the rats sampled from the 1000 mg/m^3 exposure group and in 7 of the ten rats from the 500 mg/m^3 exposure group. Most of the rats from either exposure group were graded in the +1 to +2 range. The presence of epithelial cells in JP-8 exposed rat urine was not a prominent finding at 2 weeks postexposure.

TABLE 31. EFFECT OF 90 DAY CONTINUOUS INHALATION OF JP-8 VAPORS ON RAT URINE OSMOLALITY[a]

	Unexposed Controls	500 mg/m^3 Exposed	1000 mg/m^3 Exposed
Preexposure	1448 ± 99	1462 ± 83	1413 ± 113
Exposure Termination	1005 ± 60	926 ± 141	794 ± 60[b]
2 Week Postexposure	1496 ± 114	1169 ± 57[b]	1223 ± 43[b]

[a]Mean ± S.E., N=10
[b]Statistically different from control value at $p \leq 0.05$.

Discussion

The available results of this study strongly suggest that exposure to JP-8 vapors has produced a type of toxic response similar to that seen with previously tested hydrocarbon fuels. Continuous exposure to JP-4, JP-5, and diesel fuel marine (DFM) has resulted in renal tubular necrosis in male rats. This effect has been reflected by increased BUN and creatinine levels in blood and was often accompanied by depressed red blood cell counts, hematocrit and hemoglobin

levels. Subnormal body weight gains as well as kidney weight elevations have also been noted in hydrocarbon exposed male rats. The impaired urine concentrating capability as well as the presence of epithelial cells in the urine of male rats exposed to JP-8 are further indications of renal damage. The decreased presence of epithelial cells in the urine of JP-8 exposed rats 2 weeks after termination of exposure suggests an early reversal of the nephrotoxic insult and that regeneration and repair processes are underway.

TABLE 32. INCIDENCE[a] OF EPITHELIAL CELLS IN URINE OF RATS EXPOSED TO JP-8 VAPORS CONTINUOUSLY FOR 90 DAYS

	Controls			500 mg/m^3 Exposed			1000 mg/m^3 Exposed		
Sample No.	Pre-Expo	Expos Term	2 wk Post	Pre-Expo	Expos Term	2 wk Post	Pre-Expo	Expos Term	2 wk Post
1.	0	0	tr	tr	+1	tr	0	+1	+1
2.	tr	+1	tr	0	+2	tr	0	+2	+1
3.	0	tr	tr	0	+2	tr	0	+2	0
4.	0	tr	tr	0	0	tr	0	+3	+1
5.	0	0	0	0	+3	0	0	+2	+1
6.	0	tr	0	0	tr	0	0	+1	tr
7.	0	tr	tr	0	+1	0	0	+1	+1
8.	0	tr	0	0	+2	0	0	+2	+1
9.	0	tr	tr	0	+1	tr	0	+2	0
10.	0	+1	tr	0	tr	+1	0	+1	tr

[a]Epithelial cell presence was graded on a scale of 0 to +4 with 4 being the highest incidence, greater than 20 cells per low power field. Trace amounts (tr) were scored as incidence between 0 and +1.

Histopathologic examination of the tissues collected is incomplete. Therefore, the presence and extent of renal damage in male rats is not confirmed nor have other possible sites of toxic insult of JP-8 been investigated. Results of these examinations will be discussed in future annual reports.

NEUROTOXICITY STUDIES ON FYRQUEL 220, DURAD MP280, AND HOUGHTO-SAFE 273

The U. S. Navy presently uses phosphate ester based fluids in many of its shipboard hydraulic systems. The hydraulic fluids in use are desirable because of their flame retardant characteristics. However, there is a potential health concern associated with the use of aryl ester derivatives of phosphoric acid because of the known neurotoxicity of certain members of this chemical class.

The Navy currently has a program to evaluate the engineering properties of glycol-water hydraulic fluids which, if found desirable from an engineering standpoint, would be used as candidate replacements. The water-glycol mixtures are also fire retardant.

Studies involving evaluation of the toxic effects of the various hydraulic fluids are necessary in order to properly compare the health risks associated with the various hydraulic fluids.

The Navy Medical Research Institute/Toxicology Detachment requested that the THRU conduct a series of acute and subchronic toxicity studies with three hydraulic fluids. Two of the fluids, Fyrquel 220 and Durad MP280, are phosphate ester based while Houghto-Safe 273 is water-glycol based.

Included in the series of studies was an acute delayed neurotoxicity evaluation of the hydraulic fluids. The results of the observations during the 30 day study were reported in the 1982 Annual Report (MacEwen and Vernot, 1982).

This report presents results of the histopathologic examination of the nerve tissues taken from selected hens at the conclusion of the 30-day period. Included in these studies were positive control hens exposed to triorthocresylphosphate (TOCP) for comparison standards.

No dose-related histopathologic changes were evident in the neuropil of Fyrquel 220 or corn oil treated rats. The only degenerative changes noted in these groups consisted of complete loss of randomly scattered axons believed to be related to post-mortem autolysis.

Microscopic changes observed in the Durad MP280 treated hens were identical to the changes seen in the TOCP dosed hens, although the severity of the changes in the TOCP dosed hens was greater. Axonal degeneration and demyelinization were noted in both groups and appeared to be sequential changes. The sequence probably began

with swelling of the axon resulting in myelin compression and ellipsoid formation. These events were followed by axonal fragmentation and lysis and, finally, by the complete loss of myelin. The lesions considered significantly related to Durad MP280 exposure are degeneration and demyelinization in the white matter of the spinal cord.

The results of the histopathologic examination of nerve tissue support the conclusions presented in the previous report which were based on the observations of neurotoxic signs. Durad MP280 presents a health hazard due to its neurotoxic effects, while Fyrquel 220 would be considered an acceptable fluid under the standards established by the U. S. Navy.

EVALUATION OF THE 21 DAY REPEATED DOSE DERMAL TOXICITY OF FYRQUEL 220

As part of the toxicity evaluation of various hydraulic fluids requested by NMRI/TD, repeated dose dermal exposures are being conducted using Houghto-Safe 273, Durad MP280, and Fyrquel 220. The 21 day tests with Fyrquel 220 were completed during the year. Studies of the other materials are in progress. The results will be available for the next annual report.

Male and female New Zealand white rabbits were used to evaluate the toxic hazard of repeated dermal contact with Fyrquel 220.

Groups of 10 male and 10 female rabbits weighing between 2-3 kg received occluded applications on weekdays for three consecutive weeks (15 applications). Occlusion lasted for 6 hours daily. Hair on the back was clipped from the animal as necessary with application of the material over the clipped area. Gauze patches were placed over the application area and the entire trunk of the animal was wrapped with an impervious polyethylene material held in place with elastoplast tape. Upon removal of the wrapping, the skin was wiped (not washed) in order to remove excess test material. The rabbits were restrained in stocks during dosing to prevent disturbance of the wrap.

The exposed skin area of five animals from each group was abraded once each week throughout the study. The abrasions did not penetrate the stratum corneum. Dermal irritation scores according to the method of Draize (1959) were recorded daily immediately prior to the next application of the test material.

Three dose levels were used for testing. The highest dose (5 ml/kg) was selected after preliminary studies indicated adverse effects on body weight gain could be expected. This "effect level" was also the maximum amount that could reasonably be applied to a rabbit using a 4 x 4 gauze patch (approximately 15 ml for a 3 kg rabbit). Lower doses of 0.5 and 2.5 mg/kg were chosen to establish a "no effect" level. Corn oil was used both as a diluent and vehicle for the lower doses so that the volume of liquid applied was constant. The high dose was applied as neat material. A sham control group treated with corn oil was also maintained. Due to the oily nature of the Fyrquel 220, it was impossible to remove completely all of the material at the end of the exposure period, and we expected that a certain amount would be ingested by the rabbits preening during non-exposure hours. Thus corn oil was selected as the vehicle since it provided a nontoxic soluble base for dilution so that equi-volume doses could be applied.

Body weights were measured and recorded daily prior to dosing. Toxic signs were also recorded when noted. The following hematology and blood chemistry determinations were performed at the beginning (shortly before the first application) and at the end of the 21 day study period:

RBC	Albumin
Hematocrit	Globulin
Hemoglobin	BUN
WBC	Bilirubin
Differentials	Alkaline Phosphatase
SGPT	Creatinine
SGOT	Glucose
Sodium	Cholinesterase (Serum and Erythrocyte)
Potassium	
Total Protein	

To accomodate necropsy work loads the two high dose groups were started on test one day later than the control and low dose groups. At the time of necropsy, liver, kidney, heart and spleen weights were recorded and these tissues were taken for histopathology examination along with samples of skin, ovaries, testes, thyroid, adrenals, brain, and other lesions when observed.

Results

No deaths occurred in the male rabbits treated with Fyrquel 220 but there were three deaths in exposed females. A control rabbit was killed after four exposures when it became lethargic and showed severe weight loss, nasal discharge, and diarrhea. An obstruction consisting of tightly packed ingesta was found in the upper colon. Pasteurella multocida was cultured from the nasal area of the rabbit. A female rabbit from the 0.5 ml/kg exposure group died after 9 exposures. The symptoms noted in this rabbit were similar to those of the control rabbit. Non-pathogenic E. coli was cultured from the feces. One female rabbit from the 2.5 ml/kg exposure group was removed after 9 exposures. Weight loss, diarrhea, and lethargy were also symptoms in this rabbit but the bacteriology results were negative. No intestinal obstruction was noted; however, ulcerations of the stomach were seen. No deaths occurred in the group of female rabbits treated with 5 ml/kg Fyrquel 220. None of the deaths occurring in the lower dose groups were believed to be related to Fyrquel 220 toxicity.

Skin irritation was evaluated for each rabbit at the time of dosing. Interestingly, the corn oil treated control rabbits exhibited the greatest degree of skin irritation. After the first week of dosing, many of the male and female rabbits treated with corn oil or with Fyrquel 220 at 0.5 ml/kg (10% Fyrquel 220/90% corn oil) had dry skin with exfoliation of the epidermal layer. After two weeks of dosing, well defined erythema was noted in these rabbits. The irritation was deemed so severe in three female control rabbits that the 15th and final dose was not applied. We are unable to explain this unexpected finding of irritation resulting from corn oil and have found no reference to this effect in the literature. The female control and low dose rabbits became very aggressive as the dosing regimen progressed and the aggression seemed to correlate with the skin irritation. Significant signs of skin irritation were not noted in the rabbits dosed at 2.5 ml/kg Fyrquel 220 (50% Fyrquel 220/50% corn oil) or 5 ml/kg (100% Fyrquel 220). The major symptom attributable to Fyrquel 220 exposure was the production of soft stools in both male and female rabbits dosed at 5 ml/kg. Feces of the rabbits treated with lower doses appeared normal. Occasional diarrhea was noted in the groups; however, this did not appear to be exposure related.

The effect of repeated dermal Fyrquel 220 exposure on male rabbit body weight is shown in Figure 14. As noted earlier control and low dose groups were started one day earlier than the high dose groups and weights shown in figures 14 and 15 are one day out of phase for these groups. Weight gain was retarded by dose levels of

2.5 ml/kg or greater when compared to the control or 0.5 ml/kg treatment level. Weight differences between the 5 ml/kg exposure group and control group were statistically significant ($p \leq 0.05$) when calculated using the Student t-test. During the second week of treatment, the control rabbits began to lose weight and the statistical difference between the control and 5.0 ml/kg treated rabbits disappeared. Body weights of the 2.5 ml/kg and 5.0 ml/kg treatment groups were significantly less ($p \leq 0.05$) than the 0.5 ml/kg treatment group at this time. The weight loss seen in the control rabbits corresponded to the presence of skin irritation.

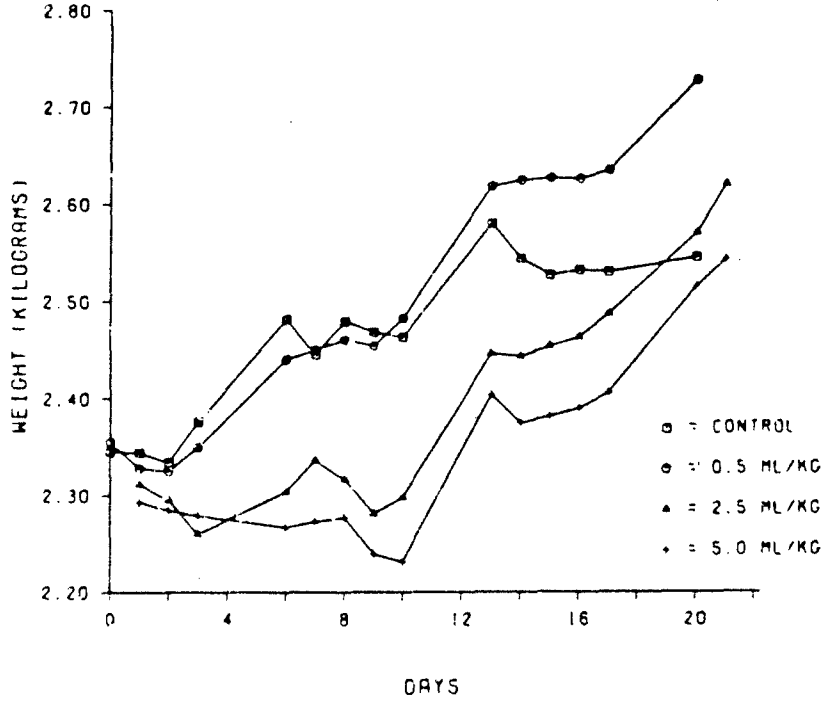


Figure 14. Effect of repeated dermal exposure to Fyrquel 220 on male rabbit body weight.

Female rabbit body weights are shown in Figure 15. None of the treated group mean body weights were found to be significantly different ($p < 0.05$) from the control group during the course of the study; however, examination of the weight curves does indicate some trends. Both the control group and 0.5 ml/kg treatment group showed general body weight gain over the course of the 21 day period. Weight loss was seen in both of these groups during the 3rd week of dosing. As with male rabbits this is thought to be a response to

the skin irritation produced by the corn oil. Female rabbits exposed to 2.5 ml/kg or 5.0 ml/kg began the study at slightly greater weights than controls. Gradual weight loss followed by gradual weight gain occurred in both of the higher dose groups over the study period. Final mean body weights of these two groups were approximately equal to their starting weights, whereas the control and the low level treatment group showed a net gain.

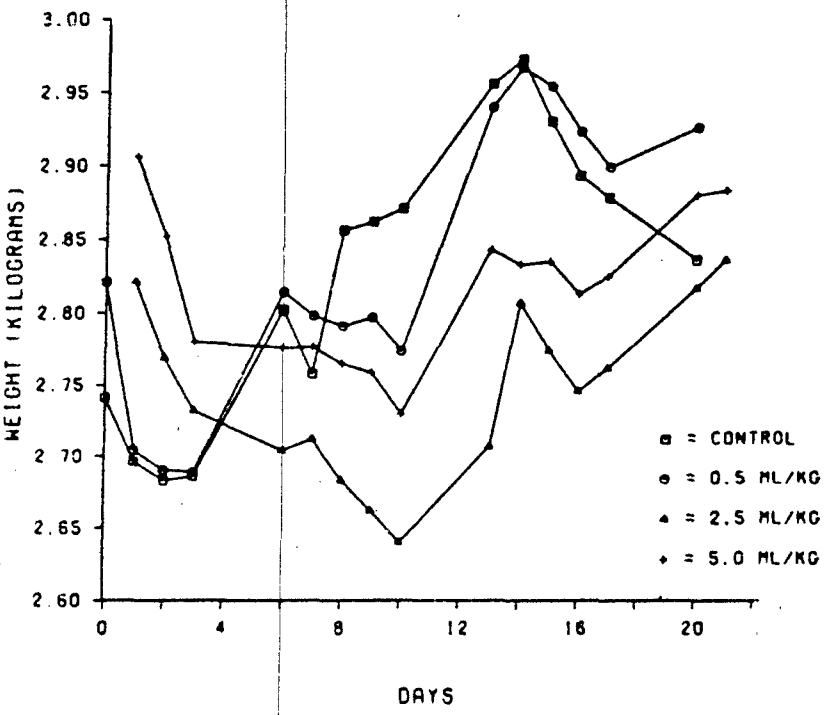


Figure 15. Effect of repeated dermal exposure to Fyrquel 220 on female rabbit body weight.

Food consumption was measured for each test group. There were no significant differences in food consumption between groups and the only reduction in food consumption seen was in the control rabbits corresponding to the skin irritation and weight loss seen during the final days of treatment with the corn oil.

In previous Fyrquel 220 aerosol inhalation studies conducted by the THRU, substantially increased rat liver weights were noted. Organ weight measurements of the rabbits treated dermally with Fyrquel 220 did not exhibit any significant increase in liver weight (Table 33). Heart, spleen, and kidney weights of Fyrquel 220 treated rabbits were also comparable to control values.

TABLE 33. EFFECT OF REPEATED DERMAL EXPOSURE TO PYRQUEL 220 ON RABBIT ORGAN WEIGHTS[a]

	Male			
Dose Level	Control	0.5 ml/kg	2.5 ml/kg	5.0 ml/kg
Body Wt (kg)	2.49 ± 0.08	2.70 ± 0.03[b]	2.54 ± 0.06	2.45 ± 0.09
Heart Wt (kg)	6.99 ± 0.49	8.61 ± 0.65[b]	6.94 ± 0.45	6.63 ± 0.41
Heart/100 g Body Wt	0.29 ± 0.03	0.32 ± 0.02	0.27 ± 0.02	0.27 ± 0.01
Liver Wt (g)	77.68 ± 3.15	86.68 ± 4.04	83.15 ± 3.69	76.38 ± 2.98
Liver/100 g Body Wt	3.13 ± 0.10	3.22 ± 0.15	3.28 ± 0.14	3.13 ± 0.12
Spleen Wt (g)	0.95 ± 0.07	1.05 ± 0.12	1.13 ± 0.08	1.05 ± 0.12
Spleen/100 g Body Wt	0.04 ± 0.003	0.04 ± 0.003	0 05 ± 0.003	0.04 ± 0.006
Kidney Wt (g)	16.22 ± 0.66	17.58 ± 0.49[c]	17.69 ± 0.30	17.25 ± 0.92
Kidney/100 g Body Wt	0.66 ± 0.03	0.65 ± 0.02[c]	0.70 ± 0.02	0.70 ± 0.02
	Female			
Dose Level	Control[c]	0.5 ml/kg[c]	2.5 ml/kg[c]	5.0 ml/kg
Body Wt (kg)	2.77 ± 0.10	2.86 ± 0.09	2.76 ± 0.12	2.77 ± 0.06
Heart Wt (g)	8.89 ± 0.25	7.89 ± 0.38[b]	7.59 ± 0.78	8.70 ± 0.95
Heart/100 g Body Wt	0.33 ± 0.02	0.28 ± 0.02	0.27 ± 0.02	0.31 ± 0.03
Liver Wt (g)	84.76 ± 4.17	85.39 ± 1.55	83.65 ± 3.72	92.09 ± 3.21
Liver/100 g Body Wt	3.08 ± 0.16	3.00 ± 0.10	3.05 ± 0.10	3.33 ± 0.11
Spleen Wt (g)	1.40 ± 0.20	1.43 ± 0.17	1.33 ± 0.14	1.08 ± 0.09
Spleen/100 g Body Wt	0.05 ± 0.01	0.05 ± 0.007	0.05 ± 0.007	0.04 ± 0.003
Kidney Wt (g)	19.41 ± 0.99	18.06 ± 0.52	19.25 ± 1.07	18.84 ± 0.44
Kidney/200 g Body Wt	0.71 ± 0.04	0.64 ± 0.04	0.70 ± 0.03	0.68 ± 0.02

[a]Mean ± S.E., N = 10
[b]Statistically different from control value $p \leq 0.05$ (Student t-test)
[c]N=9

Hematology and selected clinical chemistry values of male and female rabbits are shown in Tables 34 and 35, respectively. The majority of the serum chemistry parameters measured revealed no exposure related effects. However, significantly higher BUN and creatinine levels were seen in both male and female rabbits treated at doses of 2.5 ml/kg or higher when compared to corn oil treated controls. These elevations were also indicated by comparison of postexposure values with the respective group preexposure value. An apparent increase in the alkaline phosphatase values of male and female rabbits treated with 2.5 ml/kg or higher was noted when compared to controls, but comparison of the postexposure values of these groups with respective preexposure values indicated little change. Postexposure alkaline phosphatase levels of control rabbits were considerably less than preexposure baseline levels. The physiological significance of these alkaline phosphatase changes is not known.

TABLE 34. EFFECT OF REPEATED DERMAL EXPOSURE TO FYRQUEL 220 ON MALE RABBIT HEMATOLOGY AND BLOOD CHEMISTRY VALUES[a]

	Male							
Dose Level	Control	N	0.5 ml/kg	N	2.5 ml/kg	N	5.0 ml/kg	N
RBC (10^6 cells/mm^3)								
Preexposure	5.47 ± 0.12	10	5.48 ± 0.09	10	5.33 ± 0.11	8	5.48 ± 0.15	9
Postexposure	6.09 ± 0.18	9	6.33 ± 0.11	10	5.84 ± 0.15	10	6.15 ± 0.11	10
HCT (%)								
Preexposure	38.2 ± 0.8	10	38.1 ± 0.4	10	35.4 ± 0.7	8	38.7 ± 1.0	9
Postexposure	39.4 ± 1.0	9	41.0 ± 0.8	10	38.3 ± 1.0	10	40.4 ± 0.7	10
HGB (g/dl)								
Preexposure	12.1 ± 0.2	10	12.3 ± 0.1	10	12.0 ± 0.2	8	12.4 ± 0.3	9
Postexposure	13.2 ± 0.4	9	13.9 ± 0.3	10	13.0 ± 0.3	10	13.8 ± 0.2	10
WBC (10^3 cells/mm^3)								
Preexposure	7.5 ± 0.5	10	7.0 ± 0.3	10	7.0 ± 0.4	8	7.0 ± 0.7	9
Postexposure	10.9 ± 1.0	9	9.2 ± 0.7	10	9.3 ± 0.2	10	8.4 ± 0.4	10
Alk. Phos (IU/L)								
Preexposure	17.9 ± 2.4	8	21.8 ± 1.1	7	21.1 ± 1.1	8	22.4 ± 2.2	5
Postexposure	13.6 ± 1.3	9	17.8 ± 1.4	10	21.1 ± 1.8[b]	10	20.1 ± 1.0[b]	10
BUN (mg/dl)								
Preexposure	20.3 ± 1.2	8	17.2 ± 1.2	8	17.8 ± 1.0	5	19.1 ± 1.4	4
Postexposure	18.8 ± 1.0	9	17.0 ± 0.8	10	24.2 ± 1.0[b]	10	29.7 ± 1.0[b]	10
Creatinine (mg/dl)								
Preexposure	0.8 ± 0.04	8	0.8 ± 0.04	8	0.8 ± 0.04	5	0.8 ± 0.05	4
Postexposure	1.0 ± 0.04	8	1.0 ± 0.07	4	1.3 ± 0.03[b]	10	1.1 ± 0.1[b]	9

[a]Mean ± S.E.
[b]Statistically different from control value at p ≤ 0.05.

All groups of rabbits, whether corn oil or Fyrquel 220 treated, had mildly increased white blood cell counts at exposure termination when compared to preexposure values. This is probably a stress related response. The difference between control and Fyrquel 220 exposed white blood cell counts of female rabbits at exposure termination is probably incidental to exposure since there is no dose response. Female rabbits exposed to Fyrquel 220 tended to have elevated red blood cell counts, hematocrit and hemoglobin levels. The lack of a dose response suggests that these changes were also incidental to Fyrquel 220 exposure.

TABLE 35. EFFECT OF REPEATED DERMAL EXPOSURE TO FYRQUEL 220 ON FEMALE RABBIT HEMATOLOGY AND BLOOD CHEMISTRY VALUES[a]

	Male							
Dose Level	Control	N	0.5 ml/kg	N	2.5 ml/kg	N	5.0 ml/kg	N
RBC (10^6 cells/mm^3)								
Preexposure	5.36 ± 0.18	8	5.83 ± 0.25	8	5.42 ± 0.11	10	5.35 ± 0.11	10
Postexposure	5.56 ± 0.20	9	6.23 ± 0.16[c]	9	6.02 ± 0.15	9	6.37 ± 0.15[c]	10
HCT (%)								
Preexposure	36.0 ± 1.0	8	39.0 ± 1.3[b]	8	36.0 ± 0.66	10	35.6 ± 0.5	10
Postexposure	36.8 ± 1.4	9	41.1 ± 1.0[c]	9	39.5 ± 0.80	9	41.5 ± 0.9[c]	10
HGB (g/dl)								
Preexposure	12.1 ± 0.3	8	13.1 ± 0.5[b]	8	12.2 ± 0.2	10	12.0 ± 0.2	10
Postexposure	12.5 ± 0.5	9	13.8 ± 0.3[c]	9	13.4 ± 0.2[b]	9	14.1 ± 0.3[c]	10
WBC (10^3 cells/m^3)								
Preexposure	7.5 ± 0.6	8	4.7 ± 0.2	8	7.6 ± 0.6	10	6.7 ± 0.6	10
Postexposure	14.2 ± 1.4	9	11.4 ± 1.0	9	9.7 ± 0.8[b]	9	10.34 ± 0.6[b]	10
Alk. Phos (IU/L)								
Preexposure	15.1 ± 1.0	8	20.1 ± 1.3	9	18.5 ± 1.4	10	19.3 ± 2.2	9
Postexposure	10.4 ± 1.9	9	15.3 ± 1.8	9	14.9 ± 2.3[b]	9	19.1 ± 1.9[c]	10
BUN (mg/dl)								
Preexposure	15.4 ± 0.8	5	22.5 ± 3.8	9	16.9 ± 0.7	9	19.2 ± 1.7	9
Postexposure	16.9 ± 0.8	9	20.1 ± 1.3[c]	9	25.0 ± 1.5[c]	9	29.3 ± 1.8[c]	10
Creatinine (mg/dl)								
Preexposure	1.0 ± 0.1	4	1.0 ± 0.07	8	0.9 ± 0.04	8	1.0 ± 0.08	7
Postexposure	1.0 ± 0.1	4	1.2 ± 0.07	9	1.2 ± 0.07[b]	9	1.4 ± 0.09[b]	10

[a]Mean ± S.E.
[b]Statistically different from control value at $p \leq 0.05$.
[c]Statistically different from control value at $p \leq 0.01$.

Results of the blood cholinesterase measurements conducted by NMRI/TD have not been received. Examination of tissues from the rabbits is also not yet complete. Without these data, definitive conclusions cannot be drawn. However, the evidence available to date suggests that repeated dermal exposure to 2.5 ml/kg Fyrquel 220 can be considered an effect level with kidney dysfunction and depressed weight gain as indicators, while 0.5 ml/kg may be considered a no-effect level. The 2.5 ml/kg dose is approximately equal to the maximum cut off dose of 2 ml/kg recommended for the EPA acute dermal toxicity test and represents a relatively high level of exposure.

EVALUATION OF 21 DAY REPEATED DOSE INHALATION TOXICITY OF FIRE RESISTANT HYDRAULIC FLUIDS

The 1982 THRU Annual Report described a series of toxicity studies conducted for the U. S. Navy (Naval Medical Research Institute/Toxicology Detachment) using fire resistant hydraulic fluids (MacEwen & Vernot, 1982). Two of the hydraulic fluids being evaluated are triarylphosphates (Fyrquel 220 and Durad MP280), while the

third fluid is a water-glycol mixture (Houghto-Safe 273). Physical and chemical parameters of the three hydraulic fluids were given in the 1982 annual report (MacEwen and Vernot 1982).

As part of the toxicity evaluation requested by NMRI/TD, repeated dose, 21 day aerosol inhalation exposures were conducted.

Animals

Male and female Fischer 344 rats (8-10 weeks of age) and male Golden Syrian hamsters (8-10 weeks of age) were purchased from Charles River Breeding Laboratories, Wilmington, Mass. Male rabbits (3-4 kgs) used in the Fyrquel 220 and Durad MP280 exposures were purchased from Ancare Corporation, Manhasset, New York. Female rabbits (3-4 kgs) used in these same studies were purchased from Dutchland Labs Inc., Denver, Pennsylvania. Male and female rabbits (3-4 kgs) used in the Houghto-Safe 273 exposures were purchased from Plummers Rabbit Ranch, Peebles, Ohio.

Methods

NMRI/TD provided information of industrial hygiene surveys aboard ship which indicated maximum aerosol concentrations of 25 mg/m^3 (8 mg/m^3 average). These surveys were conducted for the glycol based materials; however, it was assumed that this level of aerosol is similar to levels produced when triarylphosphate fluids are used in similar shipboard operations. Based on these data, a level of 25 mg/m^3 was chosen as the low level exposure and 250 mg/m^3 for the high level exposure.

The experimental regimen for Fyrquel 220, Durad MP280, and Houghto-Safe 273 was identical. Groups consisted of 10 male and 10 female Fischer 344 rats, 10 male Golden Syrian hamsters, and 4 male and 4 female New Zealand white rabbits. Exposure to the hydraulic fluid aerosol was conducted on a 6 hour/day, 5 day/week basis. Exposures were not conducted on weekends. All animals were killed for tissue collection and evaluation on day 21 of the study. Fifteen exposures were conducted during the 21 day period in the Thomas Dome Inhalation Chambers.

The Fyrquel 220 and Durad MP280 studies each had sham exposed control groups of 10 male and 10 female rats and 10 male hamsters. Because of limited cage space in the chamber it was not possible to maintain separate rabbit control groups for each test material.

Therefore, a single sham exposed group of 4 male and 4 female rabbits was used for both studies. The Fyrquel 220 and Durad MP280 studies were initiated one week apart. Because of technical difficulties with the generation and chemical analysis system chosen for the Houghto-Safe 273 study, it was necessary to conduct these exposures at a later date. A separate sham exposed control group of rats, hamsters, and rabbits was maintained for this study. Animals were housed in wire mesh cages. Water was available ad libitum. Food was withheld during the 6-hour exposure but was available ad libitum during non-exposure hours.

Aerosol generation techniques for both Fyrquel 220 and Durad MP280 were similar. Collison-type nebulizers (BGI Inc., Waltham, Mass.) mounted in 250 ml distillation flasks were used to generate the hydraulic fluid aerosols. Low level exposures used a 3 jet nebulizer modified by closing off 1 jet. High level exposures used a 3 jet and a 6 jet nebulizer mounted in the same flask.

Fyrquel 220 and Durad MP280 aerosol concentrations were determined by spectrophotometric analysis of filter sample eluants. Aerosol samples were deposited on prewashed Gelman Metricel Filters (0.45 μ). The filter was mounted on a open-faced filter holder which was connected by 1/4 inch polyflow tubing to a double diaphragm vacuum pump followed by a flowmeter placed at the end of the flowtrain. The sampling system was flow-calibrated daily with a wet-test meter at a rate of 5.0 liters per minute. Flow was adjusted by means of a flow control mounted on the inlet side of the pump.

To sample the aerosol, the filter holder was inserted into the chamber through a sampling port in the window. Two minutes were allowed before starting the pump to permit equilibration of the desired concentration. High concentration aerosol was drawn for one minute producing a five liter sample volume. Filter samples for the low concentration aerosol analysis employed a 10-minute run for a 50 liter aerosol sample volume.

After sampling, the filter with its deposit of hydraulic fluid was placed in a plastic petri disk to which was added 5.0 ml reagent grade isopropyl alcohol. The filter and eluant were swirled for fifteen minutes on a platform mixer. A filter blank was prepared by elution of a pre-washed filter in the same manner.

Preliminary washing of the filters was found to be necessary due to the erratic UV response of the untreated filters. An isopropyl alcohol wash was sufficient to dilute the filters' UV activity to within an acceptable absorbance range. The procedure

consisted of immersion of one-hundred filters (one package) in about 500 ml of isopropyl alcohol. Filters were allowed to soak, with occasional swirling, for thirty minutes. Isopropyl alcohol was decanted and the procedure was repeated. Filters were then placed individually on a clean, absorbent surface until dry.

The absorbence of the eluates of Fyrquel 220 and Durad MP280 and filter blanks was read on the Spectrophotometer at a wavelength of 260 nanometers.

A second method using a TSI Aerosol Photometer was employed in the low level exposure, with instrumental output standardized on the basis of the same filter sample method. The aerosol photometer had the advantage of continuous unattended monitoring capability. The TSI Photometer operates on the principle that the intensity of scattered light is proportional to particle concentration. A photodiode measures the intensity of scattered light at a 90° angle from a focused incandescent tungsten source. The response may be read as percentage of full-scale displacement on a chart recorder which is translated into a concentration value by an experimentally determined mass concentration coefficient.

Photometers were placed adjacent to each low level exposure chamber. A straight, 11-inch long tube connected the sampling port at the base of the chamber to the sample inlet of the photometer. Another line was run from the instrument exhaust port to the chamber exhaust line to avoid the TSI pump having to draw against a chamber vacuum of 5 mm Hg.

The system was designed to alternate reading of chamber aerosol and chamber input air, the latter providing a baseline. A series of valves were synchronized to operate in a staggered on-off sequence with a power supply/timer of in-house construction. The resulting sampling cycle was twenty minutes in duration; a four minute chamber sample followed by a sixteen minute air purge. High and low concentration alarm points were established to give warning of system failure.

Houghto-Safe 273 is a water, ethylene glycol, and polyglycol mixture. The former two components substantially volatilize during aerosol generation while the non-volatile polyglycols remain in the liquid phase. Since "real life" aerosols of Houghto-Safe 273 are produced by leaks in high pressure hydraulic systems, it is reasonable to assume that the actual use situation presents an environment of both ethylene glycol vapor and polyglycol aerosol. It was therefore decided that the experimental exposure would maintain and characterize both vapor and aerosol components of Houghto-Safe 273.

Prior to exposure initiation, we established that the composition of Houghto-Safe 273 was 37.5% ethylene glycol and 15.0% polyglycol. Consequently, the 25 mg/m^3 Houghto-Safe 273 exposure level translated to 9.37 mg/m^3 ethylene glycol vapor and 3.75 mg/m^3 polyglycol aerosol. Similarly, the high level exposure at 250 mg/m^3 became 93.7 mg/m^3 ethylene glycol vapor and 37.5 mg/m^3 polyglycol aerosol.

Aerosol generation systems for both the high and low level Houghto-Safe 273 exposures employed Collison-type nebulizers mounted in a 3-neck distillation flask.

Low level exposure target concentrations of 9.37 mg/m^3 ethylene glycol and 3.75 mg/m^3 aerosol were achieved by using a modified Collison-type nebulizer operating at a back-pressure of 12 psig. The modification of this normal 3-jet nebulizer was made by soldering closed two of the fluid intake ports to make, in effect, a 1-jet nebulizer. The high level exposure required a 6 jet nebulizer operating at a back pressure of 21 psig in order to maintain the 93.7 mg/m^3 ethylene glycol and 37.5 mg/m^3 aerosol concentrations.

The metal surfaces of the chambers and introduction lines as well as water within the chamber quickly adsorbed or absorbed the ethylene glycol vapor generated from the nebulizer. We found that once these surfaces were passivated, the ethylene glycol vapor concentration remained relatively stable. Therefore, it was necessary to add additional ethylene glycol at the start-up of each 6 hour exposure to bring the ethylene glycol concentration quickly to the desired level. The amount used was decreased drastically once the desired concentration was reached. The total ethylene glycol used daily varied but averaged about 40 ml. The ethylene glycol was pumped with a polystaltic pump into two resistance coil-wrapped, glass towers. The output air flow temperature was maintained at 70 ± 3°C. Stainless steel lines from the tower output to the contaminant vents were wrapped with a heating tape.

Ethylene glycol vapors were analyzed by pulling exposure chamber air samples (filtered to remove aerosol) through a Miran 1A infrared spectrophotometer. Aerosol concentration was determined with a TSI Aerosol Photometer in a manner similar to that described previously for the Fyrquel 220 and Durad MP280 exposures.

Particle size distribution analysis was performed daily for all exposures using a high volume, 8-stage, cascade impactor. The size-segregated particles were impacted on pre-weighed aluminum collection discs and analyzed gravimetrically. Mass per stage was converted to cumulative percentage mass, then probits. Log of particle

diameter versus probit plot was generated by a weighted linear regression calculation from which mass-median diameter and geometric standard deviation were obtained. Calculations were performed by a software program developed for the Hewlett-Packard 3388A Computer/Integrater.

All animals were closely observed during the 21 day period. Individual body weights were measured twice weekly. At the conclusion of the exposure phase of the study, all animals were killed for gross examination and tissue collection for histopathologic examination (Table 36).

TABLE 36. TISSUES COLLECTED FOR HISTOPATHOLOGIC EXAMINATION OF ANIMALS EXPOSED TO HYDRAULIC FLUID AEROSOLS

Gross lesions	Heart
Tissue masses or suspect tumors and regional lymph nodes	Liver
	Spleen
Larynx	Kidneys
Trachea	Brain
Lungs and bronchi	Sciatic Nerve

Blood samples were taken from all rats and rabbits for the tests listed in Table 37.

TABLE 37. CLINICAL HEMATOLOGY AND CHEMISTRY TESTS PERFORMED ON RATS AND RABBITS EXPOSED TO HYDRAULIC FLUID AEROSOLS

Hematology	Chemistry[a]
Hematocrit	Calcium
Hemoglobin	Albumin/Globulin
RBC	Total Protein
WBC	Alkaline Phosphatase
Differentials	SGOT
Mean Corpuscular Volume (MCV)	Creatinine
Mean Corpuscular Hemoblogin (MCH)	BUN
Mean Corpuscular Hemoglobin Concentration (MCHC)	[b]Cholinesterase (Serum and Erythrocyte)

[a]Samples were sent to NMRI/TD for analysis.
[b]For Fyrquel 220 and Durad MP280 only.

Whole body, brain, liver, kidney, spleen, and heart weights were measured on all rats killed.

Data from routine animal weighing, hematology, blood chemistry, and organ weighing were analyzed for statistical significance using the Student t-test.

Results

A summary of the chemical analysis results obtained during the course of the hydraulic fluid exposures is shown in Table 38. Measured concentration mean values were very close to the desired target values for all exposures. Comparison of the measured concentrations of Fyrquel 220 and Durad MP280 with nominal values indicated an approximate 65% analytical recovery. Examination of particle size information shows that the aerosols of all three hydraulic fluids were respirable.

TABLE 38. SUMMARY OF 21 DAY EXPOSURE CONCENTRATIONS AND AEROSOL PARTICLE SIZES OF FYRQUEL 220, DURAD MP280 AND HOUGHTO-SAFE 273

	Target (mg/m^3)	Nominal[a] (mg/m^3)	Measured[b] (mg/m^3)	MMAD[c]	σg[c]
Fyrquel 220	25.00	39 ± 1.0	26.0 ± 0.3	2.3 ± 0.05	1.8 ± 0.05
Fyrquel 220	250.00	402 ± 15.0	260.0 ± 4.9	2.2 ± 0.05	2.0 ± 0.05
Durad MP280	25.00	39 ± 0.8	26.0 ± 0.3	2.3 ± 0.05	2.2 ± 0.05
Durad MP280	250.00	392 ± 8.3	251.0 ± 3.1	2.3 ± 0.05	2.1 ± 0.05
Houghto-Safe 273	25.00				
(Ethylene Glycol)	9.37	d	9.4 ± 0.28	-	-
(Aerosol)	3.75	d	3.8 ± 0.03	1.6 ± 0.05	2.0 ± 0.03
Houghto-Safe 273	250.00				
(Ethylene Glycol)	93.70	d	90.0 ± 1.0	-	-
(Aerosol)	37.50	d	38.0 ± 0.3	1.6 ± 0.03	2.0 ± 0.03

[a] Mean ± S.E., N = 15, Calculated; $\frac{\text{Total amount used per day}}{\text{Total air flow per day}}$
[b] Mean ± S.E., N = 15
[c] Mean ± S.E., N = 13 to 15
[d] Not calculated

No deaths occurred upon repeated inhalation exposure of Fyrquel 220. Transient mild diarrhea was noted in two rabbits in the 25 mg/m^3 exposure and one rabbit in the 250 mg/m^3 exposure. No other overt toxic signs were noted in any of the other species tested.

Rat body weights are shown in Table 39. The statistical difference of body weights of male rats exposed to 250 mg/m^3 Fyrquel 220 from unexposed control male rats after the sixth and ninth exposure is probably meaningless since the overall weight gains of the groups through the study were comparable. The 21 day study length was apparently too short to measure significant weight changes in hamsters. However, the absence of weight loss in either group exposed to Fyrquel 220 suggests that there was no detrimental effect on growth from this exposure. Since adult rabbits were used, it was not expected that large weight gains would be seen during the short study period. Examination of the rabbit body weights showed variability in the group weights from weighing period to weighing period. No sustained exposure related effect was indicated by these data. The Student t-test was used for all measures of significance of statistical differences between group means.

At the time of necropsy, organ weights were measured on all rats. The results are shown in Tables 40 and 41 for male and female rats, respectively. Heart, spleen, kidney, and brain weights of Fyrquel 220 exposed rats were statistically equivalent to unexposed controls at $p \leq 0.05$. Increased liver weights of approximately 18% were noted in males and females exposed to 250 mg/m^3 Fyrquel 220 when compared to control values. Exposure to 25 mg/m^3 did not produce increased liver weight in male or female rats.

Rat hematologic parameters are shown in Table 42. Slight but statistically significant increased RBC values were noted in both male and female rats exposed to 250 mg/m^3 Fyrquel 220. Hematocrit values were also elevated in female rats.

Rabbit hematologic parameters are shown in Table 43. No significant differences were noted between the Fyrquel 220 exposed and unexposed control groups.

Two female rabbits in the low level Durad MP280 exposure died shortly after exposure initiation. Gross necropsy of the rabbits revealed a purulent exudate present in the thoracic cavity characteristic of Pasteurella. Bacteriologic cultures of the exudate confirmed the presence of Pasteurella multocida. One male hamster died after 9 exposures to 250 mg/m^3 Durad MP280.

Body weights of animals exposed to Durad MP280 are shown in Table 44. The mean body weight of the male rats exposed to 250 mg/m^3 were significantly greater than the control group at the beginning of exposure. This weight difference was maintained through

the exposure period. Neither male nor female exposure groups showed any weight loss during the study. Exposure to Durad MP280 had no effect on hamster body weight gain or on rabbit body weight.

TABLE 39. EFFECT OF REPEATED 6-HOUR INHALATION OF FYRQUEL 220 AEROSOL ON ANIMAL WEIGHT[a]

Number of Exposures	Control	25 mg/m³	250 mg/m³
		Male Rats (g)	
0	160 ± 1	158 ± 2	156 ± 2
4	172 ± 3	171 ± 3	170 ± 3
6	184 ± 2	172 ± 6	169 ± 4[b]
9	188 ± 2	183 ± 6	174 ± 5[b]
11	187 ± 3	191 ± 5	185 ± 4
14	206 ± 2	201 ± 5	200 ± 5
		Female Rats (g)	
0	119 ± 3	122 ± 3	121 ± 2
4	123 ± 2	127 ± 3	126 ± 2
6	129 ± 2	129 ± 3	126 ± 2
9	131 ± 3	134 ± 3	131 ± 3
11	133 ± 2	136 ± 3	131 ± 2
14	138 ± 3	138 ± 3	137 ± 2
		Male Hamsters (g)	
0	113 ± 5	103 ± 2	100 ± 3[b]
4	108 ± 4	102 ± 2	97 ± 3[b]
6	109 ± 4	104 ± 2	100 ± 3[b]
9	110 ± 4	106 ± 3	101 ± 3
11	110 ± 4	107 ± 3	102 ± 3
14	112 ± 4	109 ± 3	103 ± 3
		Male Rabbits (kg)	
0	4.16 ± 0.18	3.90 ± 0.07	4.53 ± 0.25
4	4.15 ± 0.23	3.93 ± 0.09	4.53 ± 0.22
6	4.22 ± 0.19	4.08 ± 0.08	4.33 ± 0.20
9	4.23 ± 0.21	4.04 ± 0.10	4.55 ± 0.20
11	4.28 ± 0.20	3.95 ± 0.09	4.54 ± 0.19
14	4.24 ± 0.23	3.98 ± 0.05	4.48 ± 0.21
		Female Rabbits (kg)	
0	4.40 ± 0.25	4.50 ± 0.44	4.09 ± 0.25
4	4.42 ± 0.20	4.49 ± 0.47	4.17 ± 0.22
6	4.44 ± 0.1	4.67 ± 0.48	4.07 ± 0.25
9	4.48 ± 0.20	N/A	4.25 ± 0.18
11	4.41 ± 0.18	4.66 ± 0.45	4.32 ± 0.20
14	4.35 ± 0.15	4.71 ± 0.43	4.30 ± 0.20

[a]Mean ± S.E., N = 10 rats or hamsters/group and 4 rabbits/group.
[b]Statistically different from controls, $p < 0.05$.
N/A Not available.

The results of rat organ weight measurements taken at necropsy are shown in Tables 45 and 46 for male and female rats respectively. Increased liver weights of approximately 13% were noted in male and female rats exposed to 250 mg/m³ Durad MP280 when compared to control values. Exposure to 25 mg/m³ did not increase liver weight in male or female rats.

TABLE 40. EFFECT OF REPEATED 6-HOUR INHALATION OF FYRQUEL 220 AEROSOL ON MALE RAT ORGAN WEIGHT[a]

	Control	25 mg/m^3	250 mg/m^3
Body Weight, g	202 ± 3	199 ± 4	200 ± 5
Heart wt, g	0.71 ± 0.01	0.79 ± 0.06	0.69 ± 0.03
Heart/100 g body wt	0.35 ± 0.01	0.40 ± 0.03	0.34 ± 0.01
Liver wt, g	7.16 ± 0.26	6.81 ± 0.17	8.38 ± 0.26[b]
Liver/100 g body wt	3.55 ± 0.09	3.43 ± 0.04	4.18 ± 0.05[b]
Spleen wt, g	0.46 ± 0.02	0.47 ± 0.01	0.47 ± 0.01
Spleen/100 g body wt	0.23 ± 0.01	0.24 ± 0.01	0.23 ± 0.003
Kidney wt, g	1.64 ± 0.03	1.57 ± 0.04	1.58 ± 0.05
Kidney/100 g body wt	0.81 ± 0.01	0.79 ± 0.01	0.79 ± 0.01
Brain wt, g	1.74 ± 0.02	1.71 ± 0.02	1.71 ± 0.01
Brain/100 g body wt	0.86 ± 0.01	0.86 ± 0.02	0.86 ± 0.02

[a]Mean ± S.E., N = 10
[b]Statistically different from controls, $p \leq 0.01$.

Rat hematologic parameters are shown in Table 47. A trend toward elevated red cell counts, hematocrit, and hemoglobin values was observed in exposed rats when compared to control values. All values are within normal biological limits for the species, however.

Rabbit hematologic parameters are shown in Table 48. Trends toward elevated red cell counts, hematocrit, and hemoglobin values were seen in exposed rabbits when compared to controls. The absence of statistical significance in these values was probably due to the very small sample size, although, as with the rats, all values were within normal biological ranges.

No overt signs of toxicity were noted in the animals exposed to Houghto-Safe 273. Body weights of the animals are shown in Table 49. No significant effects due to Houghto-Safe 273 exposure are indicated by these data.

TABLE 41. EFFECT OF REPEATED 6-HOUR INHALATION OF FYRQUEL 220 AEROSOL ON FEMALE RAT ORGAN WEIGHT[a]

	Control	25 mg/m^3	250 mg/m^3
Body Weight, g	132 ± 3	133 ± 3	133 ± 2
Heart wt, g	0.51 ± 0.01	0.54 ± 0.01	0.52 ± 0.01
Heart/100 g body wt	0.39 ± 0.01	0.40 ± 0.01	0.39 ± 0.01
Liver wt, g	4.17 ± 0.11	4.14 ± 0.07	4.97 ± 0.12[b]
Liver/100 g body wt	3.16 ± 0.05	3.12 ± 0.06	3.74 ± 0.09[b]
Spleen wt, g	0.35 ± 0.01	0.36 ± 0.01	0.35 ± 0.01
Spleen/100 g body wt	0.26 ± 0.01	0.27 ± 0.01	0.26 ± 0.01
Kidney wt, g	1.11 ± 0.03	1.11 ± 0.03	1.12 ± 0.02
Kidney/100 g body wt	0.84 ± 0.01	0.83 ± 0.02	0.84 ± 0.01
Brain wt, g	1.58 ± 0.02	1.63 ± 0.03	1.60 ± 0.02
Brain/100 g body wt	1.20 ± 0.02	1.22 ± 0.03	1.21 ± 0.01

[a]Mean ± S.E., N = 10
[b]Statistically different from controls, $p \leq 0.01$.

Rat organ weights collected at exposure termination are shown in Table 50. A slight increase in spleen weights of male rats was seen; this was absent in female rats. Both of the triarylphosphate hydraulic fluids produced increased liver weights in rats exposed to aerosol concentrations of 250 mg/m^3. This response was not seen in rats exposed to the glycol-based Houghto-Safe 273.

Hematologic parameters are shown in Tables 51 and 52 for rats and rabbits respectively.

Mildly increased erythrocyte values in male rats exposed to 250 mg/m^3 were seen, but were not outside the normal variation for the species.

TABLE 42. EFFECT OF REPEATED 6-HOUR INHALATION OF FYRQUEL 220 AEROSOL ON RAT HEMATOLOGIC PARAMETERS[a]

	Male		
	Control (N = 9)	25 mg/m^3 (N = 10)	250 mg/m^3 (N = 9)
RBC (10^6)	7.76 ± 0.10	8.01 ± 0.08[b]	8.01 ± 0.07[b]
WBC (10^3)	5.9 ± 0.4	5.3 ± 0.3	5.8 ± 0.3
HCT (%)	43.2 ± 0.4	44.3 ± 0.4	44.5 ± 0.5
HGB (g/dl)	15.3 ± 0.2	15.4 ± 0.1	15.6 ± 0.2
Neut (%)	20 ± 2	18 ± 1	23 ± 3
Lympho (%)	79 ± 2	81 ± 1	75 ± 3
MCV (cu microns)	55.7 ± 0.5	55.3 ± 0.3	55.5 ± 0.3
MCH (micro-micrograms)	19.8 ± 0.4	19.2 ± 0.1	19.5 ± 0.1
MCHC (%)	35.6 ± 0.6	34.7 ± 0.3	35.1 ± 0.2

	Female		
	Control (N = 8)	25 mg/m^3 (N = 10)	250 mg/m^3 (N = 9)
RBC (10^6)	7.42 ± 0.14	7.68 ± 0.08[b]	7.74 ± 0.09[b]
WBC (10^3)	6.1 ± 0.5	5.3 ± 0.3	5.2 ± 0.4
HCT (%)	41.3 ± 0.8	44.0 ± 0.5[b]	43.4 ± 0.5[b]
HGB (g/dl)	15.1 ± 0.2	15.4 ± 0.1	15.6 ± 0.2
Neut (%)	18 ± 2	19 ± 2	19 ± 2
Lympho (%)	81 ± 2	80 ± 2	80 ± 2
MCV (cu microns)	55.7 ± 0.5	55.8 ± 0.3	56.0 ± 0.5
MCH (micro-micrograms)	20.4 ± 0.6	19.5 ± 0.2	20.1 ± 0.2
MCHC (%)	36.7 ± 0.9	34.9 ± 0.3	35.9 ± 0.3

[a]Mean ± S.E.
[b]Statistically different from controls, $p \leq 0.05$.

TABLE 43. EFFECT OF REPEATED 6-HOUR INHALATION OF FYRQUEL 220 AEROSOL ON RABBIT HEMATOLOGIC PARAMETERS[a]

	Male		
	Control (N = 4)	25 mg/m^3 (N = 3)	250 mg/m^3 (N = 3)
RBC (10^6)	5.81 ± 0.07	6.42 ± 0.32	5.70 ± 0.60
WBC (10^3)	8.8 ± 1.7	8.5 ± 1.4	13.9 ± 1.4
HCT (%)	37.9 ± 1.1	40.4 ± 1.8	36.5 ± 4.9
HGB (g/dl)	12.8 ± 0.4	13.6 ± 0.5	12.3 ± 1.8
Neut (%)	22 ± 3	34 ± 5	33 ± 11
Lympho (%)	76 ± 4	65 ± 4	66 ± 11
MCV (cu microns)	65.2 ± 1.2	62.9 ± 0.3	63.6 ± 2.4
MCH (micro-micrograms)	22.1 ± 0.5	21.4 ± 0.2	21.4 ± 1.1
MCHC (%)	33.8 ± 0.2	33.8 ± 0.2	33.6 ± 0.5

	Female		
	Control (N = 4)	25 mg/m^3 (N = 3)	250 mg/m^3 (N = 3)
RBC (10^6)	6.07 ± 0.38	6.15 ± 0.54	6.11 ± 0.56
WBC (10^3)	7.4 ± 0.8	7.2 ± 1.2	5.5 ± 0.5
HCT (%)	37.9 ± 2.9	38.7 ± 2.4	38.6 ± 3.9
HGB (g/dl)	12.9 ± 1.0	13.1 ± 0.8	12.9 ± 1.2
Neut (%)	43 ± 8	35 ± 6	24 ± 4
Lympho (%)	55 ± 7	64 ± 6	73 ± 4
MCV (cu microns)	62.3 ± 1.9	63.2 ± 1.6	63.0 ± 0.6
MCH (micro-micrograms)	21.3 ± 0.7	21.4 ± 0.8	21.3 ± 0.1
MCHC (%)	34.2 ± 0.2	33.9 ± 0.5	33.7 ± 0.3

[a]Mean ± S.E.

TABLE 44. EFFECT OF REPEATED 6-HOUR INHALATION OF DURAD MP280 AEROSOL ON ANIMAL WEIGHT[a]

Number of Exposures	Control	25 mg/m³	250 mg/m³
	Male Rats (g)		
0	174 ± 4	181 ± 17.8[c]	189 ± 3[b]
4	187 ± 4	197 ± 11.2[c]	198 ± 3[b]
6	191 ± 4	204 ± 12.1[b,c]	206 ± 3[b]
9	203 ± 4	213 ± 12.2[c]	216 ± 3[b]
11	207 ± 5	222 ± 12.8[b,c]	225 ± 4[b]
14	219 ± 5	232 ± 13.1[b,c]	235 ± 4[b]
	Female Rats (g)		
0	132 ± 2	131 ± 1	136 ± 2
4	135 ± 2	135 ± 1	136 ± 2
6	135 ± 2	137 ± 1	138 ± 2
9	142 ± 3	143 ± 1	142 ± 2
11	143 ± 3	144 ± 1	144 ± 2
14	149 ± 3	150 ± 2	149 ± 2
	Male Hamsters (g)		
0	116 ± 2	115 ± 3	113 ± 3
4	111 ± 2	113 ± 3	111 ± 3
6	112 ± 3	114 ± 3	111 ± 3
9	114 ± 3	116 ± 3	112 ± 3
11	115 ± 3	117 ± 3	115 ± 3[c]
14	115 ± 3	119 ± 3	115 ± 3[c]
	Male Rabbits (kg)		
0	4.10 ± 0.18	4.02 ± 0.20	4.30 ± 0.16
4	4.15 ± 0.22	4.14 ± 0.20	4.43 ± 0.14
6	4.22 ± 0.19	4.20 ± 0.19	4.38 ± 0.12
9	4.23 ± 0.21	4.15 ± 0.19	4.35 ± 0.15
11	4.28 ± 0.20	4.21 ± 0.21	4.44 ± 0.13
14	4.24 ± 0.23	4.22 ± 0.22	4.48 ± 0.30
	Female Rabbits (kg)		
0	4.40 ± 0.25	4.21 ± 0.44	4.03 ± 0.17
4	4.42 ± 0.20	4.58 ± 0.54[d]	3.87 ± 0.24
6	4.44 ± 0.22	4.65 ± 0.47[d]	4.01 ± 0.22
9	4.48 ± 0.20	4.75 ± 0.54[d]	4.04 ± 0.22
11	4.41 ± 0.18	4.70 ± 0.49[d]	4.05 ± 0.20
14	4.35 ± 0.18	4.81 ± 0.53[d]	4.10 ± 0.20

[a] Mean ± S.E., N = 10 rats or hamsters/group and 4 rabbits/group.
[b] Statistically different from controls, $p < 0.05$.
[c] N = 9
[d] N = 2

Discussion

The major effect noted in any of the species exposed to the hydraulic fluids was the substantially increased liver weights in rats exposed to the two triarylphosphate fluids at 250 mg/m³. Microscopic examination of the tissues from these studies has not yet been completed; therefore, the extent and nature of liver injury is unknown. Results of the blood chemistry tests conducted by NMRI/TD have not yet been made available.

TABLE 45. EFFECT OF REPEATED 6-HOUR INHALATION OF DURAD MP280 AEROSOL ON MALE RAT ORGAN WEIGHT[a]

	Control (N = 10)	25 mg/m³ (N = 9)	250 mg/m³ (N = 10)
Body Weight, g	216 ± 5	226 ± 4	232 ± 4[b]
Heart wt, g	0.74 ± 0.03	0.76 ± 0.02	0.77 ± 0.02
Heart/100 g body wt	0.33 ± 0.01	0.34 ± 0.01	0.33 ± 0.01
Liver wt, g	7.08 ± 0.21	7.61 ± 0.18	8.65 ± 0.17[c]
Liver/100 g body wt	3.27 ± 0.05	3.37 ± 0.04	3.73 ± 0.04[c]
Spleen wt, g	0.48 ± 0.01	0.48 ± 0.01	0.49 ± 0.02
Spleen/100 g body wt	0.22 ± 0.003	0.21 ± 0.01	0.21 ± 0.01
Kidney wt, g	1.62 ± 0.04	1.71 ± 0.04	1.75 ± 0.03[b]
Kidney/100 g body wt	0.75 ± 0.01	0.76 ± 0.01	0.76 ± 0.01
Brain wt, g	1.76 ± 0.04	1.75 ± 0.02	1.77 ± 0.01
Brain/100 g body wt	0.81 ± 0.01	0.78 ± 0.01[b]	0.76 ± 0.01[c]

[a]Mean ± S.E.
[b]Statistically different from controls, $p \leq 0.01$.
[c]Statistically different from controls, $p \leq 0.05$.

Adult rabbits were included in the exposures since they have proved to be susceptible to the neurotoxic effects of hydraulic fluids (Siegel et al., 1965). The neurotoxic potential of Durad MP280 has been established in the chicken, while Fyrquel was found to be non-neurotoxic (MacEwen and Vernot, 1982). Obvious cholinergic symptoms were not evident in the rabbits exposed to Durad MP280. Deaths occurring in those exposures were determined to be unrelated to the exposure to the test material. Results of rat and rabbit blood cholinesterase measurements have not been forwarded by NMRI/TD. Therefore, the effect of the triarylphosphate hydraulic fluids on this enzyme is unknown. Microscopic examination of nerve tissue is also incomplete at this time.

TABLE 46. EFFECT OF REPEATED 6-HOUR INHALATION OF DURAD MP280 AEROSOL ON FEMALE RAT ORGAN WEIGHT[a]

	Control (N = 10)	25 mg/m^3 (N = 9)	250 mg/m^3 (N = 10)
Body Weight, g	144 ± 3	142 ± 1	146 ± 2
Heart wt, g	0.53 ± 0.01	0.52 ± 0.01	0.51 ± 0.01
Heart/100 g body wt	0.36 ± 0.01	0.37 ± 0.01	0.35 ± 0.01
Liver wt, g	4.39 ± 0.14	4.29 ± 0.09	5.03 ± 0.12[c]
Liver/100 g body wt	3.04 ± 0.06	3.02 ± 0.04	3.45 ± 0.06[c]
Spleen wt, g	0.38 ± 0.01	0.37 ± 0.01	0.39 ± 0.01
Spleen/100 g body wt	0.26 ± 0.003	0.26 ± 0.01	0.26 ± 0.01
Kidney wt, g	1.19 ± 0.03	1.09 ± 0.02[b]	1.21 ± 0.03
Kidney/100 g body wt	0.82 ± 0.01	0.77 ± 0.02[b]	0.83 ± 0.01
Brain wt, g	1.64 ± 0.01	1.65 ± 0.02	1.66 ± 0.01
Brain/100 g body wt	1.14 ± 0.03	1.16 ± 0.22	1.14 ± 0.02

[a]Mean ± S.E.
[b]Statistically different from controls, $p \leq 0.01$.
[c]Statistically different from controls, $p \leq 0.05$.

TABLE 47. EFFECT OF REPEATED 6-HOUR INHALATION OF DURAD MP280 AEROSOL ON RAT HEMATOLOGIC PARAMETERS[a]

	Male		
	Control (N = 8)	25 mg/m^3 (N = 7)	250 mg/m^3 (N = 10)
RBC (10^6)	7.75 ± 0.10	7.74 ± 0.14	8.43 ± 0.09[b]
WBC (10^3)	4.9 ± 0.2	4.9 ± 0.2	5.3 ± 0.2
HCT (%)	41.9 ± 0.6	42.5 ± 0.7	45.5 ± 0.5[b]
HGB (g/dl)	15.5 ± 0.1	15.9 ± 0.2	16.2 ± 0.2[b]
Neut (%)	18 ± 1	24 ± 3	21 ± 2
Lympho (%)	81 ± 1	75 ± 4	78 ± 2
MCV (cu microns)	54.1 ± 0.4	54.9 ± 0.3	54.0 ± 0.3
MCH (micro-micrograms)	20.0 ± 0.2	20.5 ± 0.3	19.3 ± 0.1[c]
MCHC (%)	37.0 ± 0.6	37.4 ± 0.5	35.7 ± 0.2[c]

	Female		
	Control (N = 8)	25 mg/m^3 (N = 8)	250 mg/m^3 (N = 9)
RBC (10^6)	7.09 ± 0.13	7.56 ± 0.16[c]	7.57 ± 0.11[b]
WBC (10^3)	5.1 ± 0.4	4.3 ± 0.3[c]	4.8 ± 0.2
HCT (%)	38.5 ± 0.7	41.4 ± 1.0[c]	40.9 ± 0.7[c]
HGB (g/dl)	14.9 ± 0.1	15.4 ± 0.2[c]	15.1 ± 0.2
Neut (%)	18 ± 2	16 ± 2	16 ± 2
Lympho (%)	80 ± 2	84 ± 3	83 ± 2
MCV (cu microns)	54.2 ± 0.3	54.8 ± 0.3	53.9 ± 0.3
MCH (micro-micrograms)	21.1 ± 0.4	20.4 ± 0.3	19.9 ± 0.2[c]
MCHC (%)	38.9 ± 0.7	37.2 ± 0.6	37.0 ± 0.5[c]

[a]Mean ± S.E.
[b]Statistically different from controls, $p \leq 0.01$.
[c]Statistically different from controls, $p \leq 0.05$.

TABLE 48. EFFECT OF REPEATED 6-HOUR INHALATION OF DURAD MP280 AEROSOL ON RABBIT HEMATOLOGIC PARAMETERS[a]

	Male		
	Control (N = 4)	25 mg/m^3 (N = 3)	250 mg/m^3 (N = 3)
RBC (10^6)	5.81 ± 0.07	6.55 ± 0.09[b]	6.55 ± 0.53
WBC (10^3)	8.8 ± 1.7	9.3 ± 2.5	11.7 ± 0.8
HCT (%)	37.9 ± 1.1	41.8 ± 0.6	43.6 ± 2.6[c]
HGB (g/dl)	12.8 ± 0.4	14.1 ± 0.3	14.9 ± 0.8[c]
Neut (%)	22 ± 3.4	33 ± 7.6	33 ± 3.7
Lympho (%)	76 ± 3.9	66 ± 6.8	65 ± 3.3
MCV (cu microns)	65.2 ± 1.2	63.8 ± 0.4	66.8 ± 2.3
MCH (micro-micrograms)	22.1 ± 0.5	21.5 ± 0.4	22.8 ± 0.8
MCHC (%)	33.8 ± 0.2	33.8 ± 0.4	34.1 ± 0.4

	Female		
	Control (N = 4)	25 mg/m^3 (N = 2)	250 mg/m^3 (N = 3)
RBC (10^6)	6.07 ± 0.38	6.20 ± 0.29	6.99 ± 0.13
WBC (10^3)	7.4 ± 0.8	6.5 ± 1.3	6.9 ± 1.1
HCT (%)	37.9 ± 2.9	39.2 ± 0.3	44.1 ± 0.5
HGB (g/dl)	12.9 ± 1.0	13.5 ± 0.3	15.0 ± 0.2
Neut (%)	43 ± 7.5	46 ± 4.5	25 ± 4.0
Lympho (%)	55 ± 7.1	53 ± 3.0	73 ± 3.7
MCV (cu microns)	62.3 ± 1.9	63.3 ± 2.5	63.1 ± 0.8
MCH (micro-micrograms)	21.3 ± 0.7	21.7 ± 0.6	21.4 ± 0.3
MCHC (%)	34.2 ± 0.2	34.3 ± 0.4	33.9 ± 0.06

[a]Mean ± S.E.
[b]Statistically different from controls, $p \leq 0.01$.
[c]Statistically different from controls, $p \leq 0.05$.

TABLE 49. EFFECT OF REPEATED 6-HOUR INHALATION OF HOUGHTO-SAFE 273 AEROSOL ON ANIMAL BODY WEIGHT[a]

Number of Exposures	Control	25 mg/m^3	250 mg/m^3
		Male Rats (g)	
0	171 ± 1	174 ± 3	172 ± 3
4	182 ± 2	188 ± 3	183 ± 3
6	179 ± 4	186 ± 3	186 ± 3[b]
9	178 ± 4	191 ± 4[b]	187 ± 4[b]
11	188 ± 4	197 ± 4	194 ± 3
14	193 ± 4	204 ± 4[b]	203 ± 3
		Female Rats (g)	
0	125 ± 2	123 ± 1	128 ± 2
3	129 ± 2	130 ± 1	132 ± 2
5	131 ± 2	130 ± 1	133 ± 2
8	131 ± 2	132 ± 2	136 ± 2
10	138 ± 2	132 ± 2	136 ± 3
13	143 ± 3	140 ± 1	141 ± 3
		Male Hamsters (g)	
0	105 ± 3	110 ± 2	112 ± 2
3	103 ± 3	107 ± 3	109 ± 2[b]
6	104 ± 3	110 ± 3	112 ± 2[b]
8	106 ± 3	110 ± 3	111 ± 3
10	109 ± 3	112 ± 3	112 ± 3
14	110 ± 3	114 ± 3	114 ± 3
		Male Rabbits (kg)	
0	4.19 ± 0.11	4.18 ± 0.08	4.52 ± 0.06[b]
4	4.37 ± 0.10	4.40 ± 0.07	4.78 ± 0.06[b]
7	4.39 ± 0.11	4.40 ± 0.08	4.70 ± 0.10[b]
9	4.40 ± 0.11	4.38 ± 0.09	4.76 ± 0.06[b]
12	4.45 ± 0.11	4.46 ± 0.09	4.77 ± 0.05[b]
14	4.43 ± 0.14	4.42 ± 0.12	4.77 ± 0.06
		Female Rabbits (kg)	
0	4.00 ± 0.14	4.17 ± 0.08	4.03 ± 0.22
4	4.15 ± 0.17	4.30 ± 0.13	4.17 ± 0.26
7	4.12 ± 0.16	4.31 ± 0.23	4.11 ± 0.21
9	4.15 ± 0.18	4.27 ± 0.08	4.19 ± 0.24
12	4.21 ± 0.19	4.45 ± 0.11	4.25 ± 0.23
14	4.25 ± 0.22	4.33 ± 0.15	4.27 ± 0.22

[a]Mean ± S.E., N = 10 rats or hamsters/group and 4 rabbits/group.
[b]Statistically different from controls, $p \leq 0.05$.

TABLE 50. EFFECT OF REPEATED 6-HOUR INHALATION OF HOUGHTO-SAFE 273 ON RAT ORGAN WEIGHT[a]

MALE

	Control	25 mg/m³	250 mg/m³
Body Weight, g	189 ± 4	198 ± 4	194 ± 3
Heart wt, g	0.65 ± 0.02	0.67 ± 0.02	0.65 ± 0.02
Heart/100 g body wt	0.35 ± 0.01	0.34 ± 0.01	0.33 ± 0.01
Liver wt, g	6.56 ± 0.19	6.58 ± 0.18	6.39 ± 0.14
Liver/100 g body wt	3.47 ± 0.08	3.32 ± 0.04	6.29 ± 0.03
Spleen wt, g	0.43 ± 0.01	0.49 ± 0.01[b]	0.49 ± 0.01[b]
Spleen/100 g body wt	0.23 ± 0.003	0.25 ± 0.003[b]	0.25 ± 0.003[b]
Kidney wt, g	1.43 ± 0.03	1.47 ± 0.03	1.42 ± 0.03
Kidney/100 g body wt	0.76 ± 0.01	0.74 ± 0.01	0.73 ± 0.01
Brain wt, g	1.69 ± 0.03	1.72 ± 0.02	1.68 ± 0.02
Brain/100 g body wt	0.89 ± 0.01	0.87 ± 0.02	0.87 ± 0.01

FEMALE

	Control	25 mg/m³	250 mg/m³
Body Weight, g	135 ± 3	133 ± 1	137 ± 2
Heart wt, g	0.50 ± 0.01	0.48 ± 0.01	0.49 ± 0.01
Heart/100 g body wt	0.37 ± 0.01	0.36 ± 0.01	0.36 ± 0.01
Liver wt, g	4.03 ± 0.10	3.89 ± 0.05	3.93 ± 0.11
Liver/100 g body wt	2.99 ± 0.05	2.92 ± 0.03	2.87 ± 0.04
Spleen wt, g	0.38 ± 0.01	0.36 ± 0.01	0.37 ± 0.01
Spleen/100 g body wt	0.28 ± 0.01	0.27 ± 0.01	0.27 ± 0.003
Kidney wt, g	1.04 ± 0.02	1.02 ± 0.02	1.03 ± 0.03
Kidney/100 g body wt	0.78 ± 0.01	0.76 ± 0.02	0.75 ± 0.01
Brain wt, g	1.62 ± 0.02	1.58 ± 0.03	1.64 ± 0.02
Brain/100 g body wt	1.20 ± 0.02	1.19 ± 0.02	1.20 ± 0.02

[a]Mean ± S.E., N = 10
[b]Statistically different from controls, $p \leq 0.01$.

TABLE 51. EFFECT OF 6-HOUR INHALATION EXPOSURE TO HOUGHTO-SAFE 273 ON RAT BLOOD[a]

MALE

Exposure Concentration	Control (N = 10)	25 mg/m^3 (N = 9)	250 mg/m^3 (N = 10)
RBC (10^6 cells/m^3)	8.25 ± 0.11	8.10 ± 0.7	8.70 ± 0.12[b]
WBC (10^3 cells/m^3)	5.1 ± 0.1	4.3 ± 0.2[c]	5.8 ± 0.3[c]
HCT (%)	43.4 ± 0.7	42.8 ± 0.4	46.0 ± 0.8[b]
HGB (g/dl)	14.5 ± 0.2	14.1 ± 0.1	15.2 ± 0.2[c]
MCV (cu microns)	52.6 ± 0.3	52.8 ± 0.2	52.9 ± 0.3
MCH (micromicrograms)	17.6 ± 0.1	17.4 ± 0.1[c]	17.4 ± 0.1[c]
MCHC (%)	33.5 ± 0.2	33.0 ± 0.2[c]	32.9 ± 0.1[b]

FEMALE

Exposure Concentration	Control (N = 8)	25 mg/m^3 (N = 10)	250 mg/m^3 (N = 9)
RBC (10^6 cells/m^3)	7.78 ± 0.14	8.01 ± 0.15	7.95 ± 0.08
WBC (10^3 cells/m^3)	5.2 ± 0.4	5.4 ± 0.3	6.1 ± 0.2[c]
HCT (%)	41.7 ± 0.7	42.7 ± 0.9	42.1 ± 0.5
HGB (g/dl)	14.7 ± 0.3	15.3 ± 0.3	15.4 ± 0.2
MCV (cu microns)	53.6 ± 0.2	53.3 ± 0.2	52.9 ± 0.2[c]
MCH (micromicrograms)	18.9 ± 0.2	19.1 ± 0.2	19.4 ± 0.2
MCHC (%)	35.3 ± 0.4	35.9 ± 1.5	36.6 ± 0.5

[a]Mean ± S.E.

[b]Statistically different from controls at $p \leq 0.01$.

[c]Statistically different from controls at $p \leq 0.05$.

TABLE 52. EFFECT OF 6-HOUR INHALATION EXPOSURE TO HOUGHTO-SAFE 273 ON RABBIT BLOOD[a]

MALE

	Control (N = 4)	25 mg/m^3 (N = 4)	250 mg/m^3 (N = 4)
RBC (10^6 cells/m^3)	5.87 ± 0.33	6.07 ± 0.11	6.45 ± 0.15
WBC (10^3 cells/m^3)	6.7 ± 0.6	9.5 ± 1.1[b]	8.2 ± 0.7
HCT (%)	39.5 ± 1.7	39.5 ± 1.2	42.5 ± 1.0
HGB (g/dl)	14.2 ± 0.4	13.7 ± 0.4	14.7 ± 0.3
MCV (cu microns)	67.4 ± 1.6	65.1 ± 1.1	65.8 ± 0.7
MCH (micro-micrograms)	24.4 ± 1.3	22.6 ± 0.4	22.7 ± 0.6
MCHC (%)	36.1 ± 1.4	34.7 ± 0.2	34.5 ± 0.6

FEMALE

	Control (N = 3)	25 mg/m^3 (N = 4)	250 mg/m^3 (N = 4)
RBC (10^6 cells/m^3)	5.58 ± 0.36	6.05 ± 0.18	6.04 ± 0.20
WBC (10^3 cells/m^3)	7.0 ± 0.9	7.2 ± 0.9	8.2 ± 0.9
HCT (%)	36.9 ± 2.2	39.7 ± 1.3	39.1 ± 1.2
HGB (g/dl)	13.7 ± 0.5	14.4 ± 0.4	14.0 ± 0.4
MCV (cu microns)	66.1 ± 0.3	65.6 ± 0.4	64.8 ± 0.3[b]
MCH (micro-micrograms)	24.7 ± 1.0	23.8 ± 0.2	23.2 ± 0.2
MCHC (%)	37.3 ± 1.4	36.3 ± 0.2	35.9 ± 0.2

[a]Mean ± S.E.
[b]Statistically different from controls at $p \leq 0.05$.

EVALUATION OF 90 DAY CONTINUOUS INHALATION EXPOSURE TO FYRQUEL 220, DURAD MP280, AND HOUGHTO-SAFE 273

Subchronic toxicity studies were conducted with three hydraulic fluids as requested by NMRI/TD. Two of the fluids, Fyrquel 220 and Durad MP280, are phosphate ester based while the third, Houghto-Safe 273, is water-glycol based.

In 1965, Siegel et al. reported the effect of long-term inhalation exposure to triarylphosphate (TAP) hydraulic fluid. The material contained a mixture of tricresylphosphates, trixylenylphosphates, and other trialkyphenylphosphates. A number of animal species were exposed continuously to TAP concentrations ranging from 1.8 mg/m^3 to 110 mg/m^3. Generalized hind limb paralysis was first noted in rabbits exposed to 102 and 103 mg/m^3 TAP. This paralysis was noted at 19 days, became progressively worse, and was accompanied by marked weight loss. All rabbits died by day 52 of exposure. Rats exposed to the same concentration exhibited no signs of neurotoxicity, and no deaths occurred.

Rabbits, rats, and hamsters were included in the present study because of the apparent species variation in sensitivity to organophosphate neurotoxicity. In addition, a number of nervous system screening tests were incorporated into the protocol to evaluate possible neurotoxicity.

NMRI/TD had provided information of industrial hygiene surveys aboard ship with indicated aerosol concentrations of 25 mg/m^3 maximum (8 mg/m^3 average). These surveys were conducted for the glycol-based materials; however, it is reasonable to assume that this level of aerosol is similar to levels produced when triarylphosphate fluids are used in similar shipboard operations. Based on these data, a continuous exposure to 10 mg/m^3 was chosen as the low level exposure and 100 mg/m^3 as the high level exposure. The low level concentrations simulated shipboard conditions while the high concentration might be expected to provide an effect level.

The experimental regimen for Fyrquel 220, Durad MP280, and Houghto-Safe 273 was identical. Male and female Fischer 344 rats, male Golden Syrian hamsters, and male and female New Zealand white rabbits were exposed to hydraulic fluid aerosol on a continuous basis for 90 days in Thomas Dome inhalation chambers. A sham exposed control group was also maintained in an inhalation exposure chamber.

Each chamber housed 20 male and 20 female Fischer 344 rats, 20 male Golden Syrian hamsters, and 4 male and 4 female New Zealand rabbits.

Exposure was uninterrupted with cage cleaning, animal feeding, and clinical testing being conducted during exposure. All animals were killed for tissue collection and examination at the conclusion of the 90 day study.

Generation techniques for both Pyrquel 220 and Durad MP280 were similar. The aerosol for the low level, 10 mg/m^3, chambers was produced by one-jet Collison nebulizers while the aerosol for the 100 mg/m^3 was produced using three-jet Collison nebulizers.

Houghto-Safe 273 was generated for the low concentration (10 mg/m^3) exposure using a Laskin single jet nebulizer. A six-jet Collison nebulizer was used for generation of the high concentration (100 mg/m^3) Houghto-Safe 273 exposure.

Houghto-Safe 273 concentrations were controlled by measuring ethylene glycol concentrations. In attempting to maintain the initial constituent proportions present in Houghto-Safe 273, it was necessary to add an auxiliary ethylene glycol vapor generation system. The ethylene glycol was pumped into a heated glass evaporator tower with a countercurrent of air carrying the vaporized ethylene glycol into the chamber input air supply.

Concentrations of Pyrquel 220 and Durad MP280 were monitored using a TSI Aerosol Photometer, Model 5150. Automatic timers allowed for four-minute samples to be taken from each chamber, three times each hour. Chamber input air during the remaining time periods established baseline values.

The volatile ethylene glycol was analyzed with a Miran IA infrared spectrometer. The chamber sample (5 L/min) was first pulled through a midget impinger and a 0.45 µm Metricel 6A filter to remove the aerosol. The Miran IR was calibrated using standard ethylene glycol bags.

Impactor samples from each chamber were taken for particle size analysis on a daily basis. Nominal sampling times were sufficient to collect a measurable amount of material. Aluminum stage collector plates were weighed before and after sampling and the mass of each calculated. The particle size mass median diameter and standard geometric deviation were calculated from these data.

All animals were observed hourly during the 90 days and were individually weighed weekly. Statistical comparisons of means were performed using the Student t-test.

Five male and five female rats from the high dose level of Durad MP280 and Pyrquel 220 as well as the control group underwent a sensory/motor screening at 2, 4, and 8 weeks, and at termination of exposure as shown below.

1. Autonomic Signs: As each rat was taken from its home cage, it was evaluated for the presence of diarrhea, lacrimation (including chromodacryorrhea), nasal discharge, salivation, exophthalmus, urinary incontinence and alopecia. Presence of any of these were noted on the record form; and absence of all autonomic signs was noted with a zero (Irwin, 1968).

2. Tail Tip Curl Reflex: Holding the rat in the right hand under the forelimbs, the tail was stroked toward the tail tip with the left forefinger beginning at a point about one third of its length from the tip, and hesitating at a point about 1 to 1 1/2 inches from the tip. In normal rats, the tail tip curled, more or less strongly, around the finger. The strength of the response was rated on a scale of 0 (no response), 1 (present but not strong/brisk), or 2 (strongly curled, brisk response). If a rat began to struggle, it was returned to its cage, then retested. A maximum of three attempts were allowed.

3. Hind Foot Position/Foot Drop: Normal rats supported under the forelimbs typically show one of two hind limb positions: (a) the feet are drawn up against the body, the ankles are flexed, the legs parallel, and the toes are extended in a relatively relaxed fashion; or (b) the legs are splayed outward, the ankles are extended in a relaxed manner or are spread. Drooping feet, crossed legs, and/or curled toes are abnormal (Snyder and Braun, 1977). Hind limb position are scored 0 (sign absent) or 1 (sign present). Scores were recorded.

4. Lateral Hop: The rat was held around the thorax in one hand and the rat's tail taken in the other. One hind foot was placed on the edge of the bench top while the tail was pulled downward slightly to put weight on the foot. When the rat was tilted in the direction of the foot in contact with the bench top, the rat hopped on that one foot in that direction. The test was done both to the left and right and the response was scored 0 (no hop or depressed response) or 1 (present/normal response) (Marshall and Teitelbaum, 1974).

Blood samples were taken for hematologic and clinical chemistry determinations from male and female rats of each exposure group. Cholinesterase determinations were made by NMRI/TD on male and

female rats and all rabbits from the high dose of Fyrquel 220, Durad MP280, and control groups. The blood clinical determinations are shown in Table 53.

TABLE 53. CLINICAL HEMATOLOGY AND CHEMISTRY TESTS PERFORMED ON MALE AND FEMALE RATS EXPOSED TO HYDRAULIC FLUIDS FOR 90 DAYS

Hematology	Chemistry
Hematocrit	Calcium
Hemoglobin	Albumin/Globulin
RBC	Total Protein
WBC	Alkaline Phosphatase
Differentials	SGOT
Mean Corpuscular Volume (MCV)	Creatinine
Mean Corpuscular Hemoglobin (MCH)	BUN
Mean Corpuscular Hemoglobin Concentration (MCHC)	Cholinesterase

All animals that died or were killed in these studies were necropsied. The necropsy was an external examination including body orifices and fixation of all of the tissues shown in Table 54. Histopathologic examination will be made on the tissues listed in Column 1. The tissues which appear in Column 2 were collected and stored for future examination if desired.

Sections of spinal cord were harvested from two rabbits and three rats of each sex exposed to 100 mg/m^3 of Fyrquel 220 and Durad MP280 plus controls. Specimens were obtained from the cervical, thoracic, and lumbar segments of each cord.

Both sciatic nerves were collected from each rabbit submitted for spinal cord removal. Dissection of sciatic nerves included distal branches extending as far below the hock joint as practical. The right sciatic nerve was placed in appropriate fixatives for electron microscopy and the left nerve fixed in 10% buffered formalin for examination by light microscopy.

The rats were fasted 18 hours prior to necropsy. At necropsy, whole body, brain, liver, kidney, spleen, and heart weights were recorded. Brains from 10 rats per high level and control groups were frozen in liquid nitrogen and sent to NMRI/TD for cholinesterase determinations.

TABLE 54. TISSUES SAMPLED FROM ANIMALS CONTINUOUSLY EXPOSED TO HYDRAULIC FLUID FOR 90 DAYS

Column 1	Column 2
Gross Lesions	Parathyroids
Tissue masses or suspect tumors and regional lymph nodes	Esophagus
Larynx	Sternebrae, vertebrae, or femur (plus marrow)
Trachea	Mandibular lymph node
Lungs and bronchi	Mammary gland
Heart	Stomach
Thyroid	Duodenum
Liver	Ileum
Spleen	Colon
Kidney	Anus
Bladder	Mesenteric lymph nodes
Nasal cavity	Thigh muscle
Brain	Thymus
Skin	Seminal vesicles
Gall bladder	Prostrate
Pancreas	Uterus
Adrenals	
Pituitary	
Testes/ovaries	
Sciatic nerve (proximal and distal sections, rabbits and rats)	
Spinal cord (rabbits and rats)	

Mean concentrations of the hydraulic fluid aerosols are shown in Table 55. The Houghto-Safe 273 concentrations are expressed as mg/m^3 of ethylene glycol which would be representative of aerosol concentrations of 100 and 10 mg/m^3 of the mixture.

The mean mass median diameter of the droplets for all of the hydraulic fluids was less than 2.5 microns. The standard geometric mean diameter was approximately 2 microns.

Natural deaths among hamsters and rats were few and sporadic (Table 56). Several deaths occurred, particularly in the high level Durad MP280 female rat group during an interim bleeding regimen. The bleeding technique was slightly altered and no further deaths occurred.

TABLE 55. MEAN CONCENTRATIONS OF HYDRAULIC FLUIDS MEASURED DURING THE 90 DAY AEROSOL STUDY

Hydraulic Fluid	Mean Conc., (mg/m^3)	S.D.	MMAD	σg
Ethylene Glycol	4.0[a]	1.0	1.1	2.0
	36.3[b]	2.7	1.6	2.0
Durad MP280	10.3	0.5	1.9	1.8
	101.0	4.7	2.0	1.9
Fyrquel 220	10.1	0.7	2.3	1.9
	100.0	4.2	2.3	1.9

[a]Equivalent to 10.4 mg/m^3 Houghto-Safe 273.
[b]Equivalent to 97.9 mg/m^3 Houghto-Safe 273.

TABLE 56. ANIMAL MORTALITY DURING EXPOSURE TO HYDRAULIC FLUIDS

		% Mortality					
		Fyrquel 220		Durad MP280		Houghto-Safe 273	
Species and Sex	Control	10 mg/m^3	100 mg/m^3	10 mg/m^3	100 mg/m^3	10 mg/m^3	100 mg/m^3
Rats, Male (20)	5.0	0.0	0.0	0.0	0.0	0.0	0.0
Rats, Female (20)	10.0	0.0	5.0	0.0	25.0	0.0	5.0
Hamsters, Male (20)	0.0	0.0	0.0	5.0	0.0	0.0	5.0
Rabbits, Male (4)	0.0	0.0	25.0	0.0	100.0[a]	0.0	25.0
Rabbits, Female (4)	25.0	0.0	50.0[a]	25.0[a]	100.0	0.0	0.0

( ) = Original number.
[a] = Includes a death from pasteurella multocida infection.

Signs of toxic stress were noted in the high level Durad MP280 rabbit group as early as three days after exposure initiation. The rabbits became anorexic and lethargic. This was followed by cachexia accompanied by head droop prior to death. Hind limb paralysis was not noted. All rabbits from this group had died by the 49th exposure day.

Rabbit mortality occurred in several of the other groups, including controls. Pasteurella multocida was the cause in some of these deaths, including one rabbit that died from the low level Durad MP280 group.

Kyphosis was noted in rats early in the 100 mg/m^3 Durad MP280 exposure. This persisted throughout the 90 days. To a lesser

extent, kyphosis was also noted in the rats exposed to 100 mg/m^3 Fyrquel 220. These groups of rats also had rough coats and a general unkempt appearance.

Exposure to Fyrquel 220 at either concentration had no effect on the body weight gain of male rats (Figure 16) or male hamsters (Figure 17). Depressed weight gains were noted in female rats exposed to 100 mg/m^3 while 10 mg/m^3 had no effect (Figure 18).

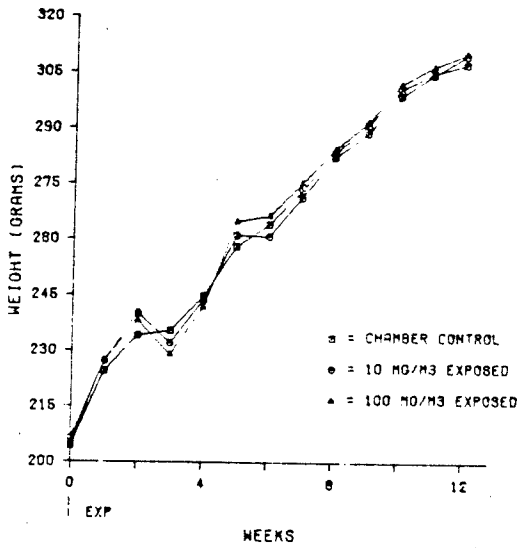


Figure 16. Effect of Fyrquel 220 exposure on male rat body weight.

Both sexes of rats exposed to Durad MP280 had significantly lower weight gains than respective controls (Figures 19 and 20). Female rats exposed to 10 mg/m^3 exhibited an erratic growth curve that was generally lower than, but not statistically different from the controls. Hamsters exposed to 100 mg/m^3 also showed depressed weight gain particularly during the latter stages of the exposure when weight loss occurred (Figure 21).

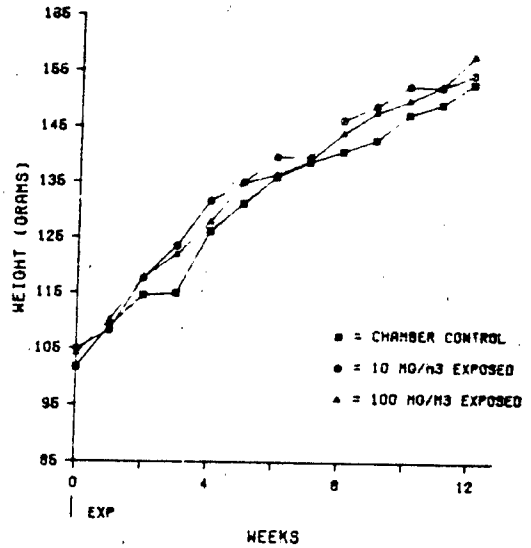


Figure 17. Effect of Fyrquel 220 exposure on male hamster body weight.

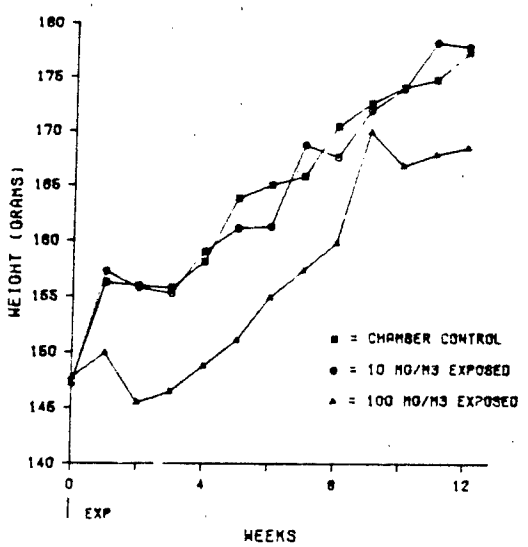


Figure 18. Effect of Fyrquel 220 exposure on female rat body weight.

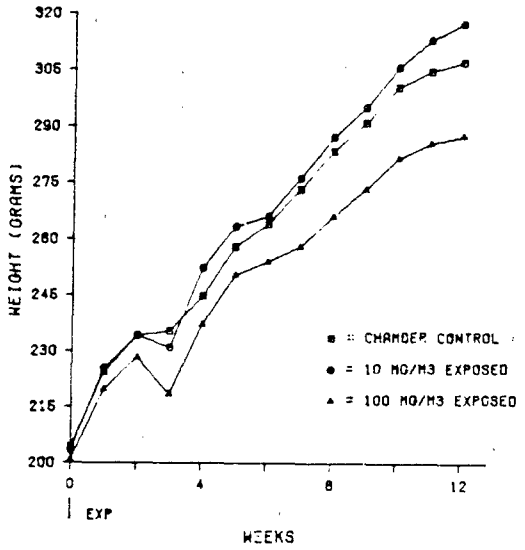


Figure 19. Effect of Durad MP280 exposure on male rat body weight.

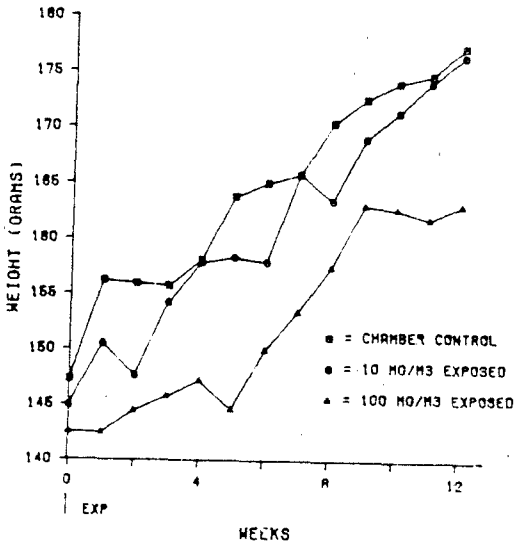


Figure 20. Effect of Durad MP280 exposure on female rat body weight.

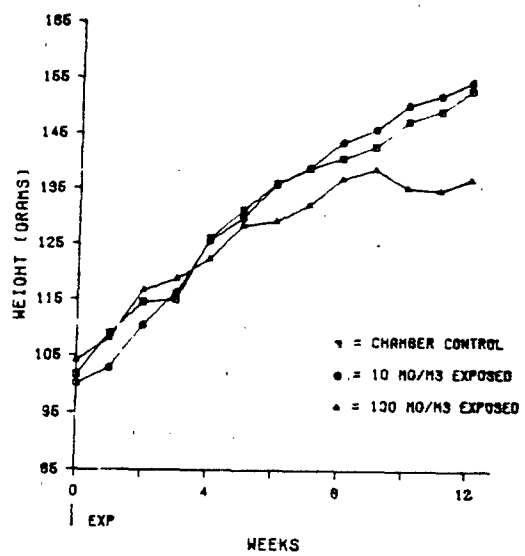


Figure 21. Effect of Durad MP280 exposure on male hamster body weight.

Male rats and hamsters were unaffected by exposure to Houghto-Safe 273 at either concentration (Figures 22 and 23). Female rats exposed to 100 mg/m^3 (Figure 24) had depressed weight gains compared to controls while the lower exposure concentration did not affect growth.

Results of the CNS screening examination of rats are shown in Table 57. Neither Fyrquel 220 nor Durad MP280 substantially altered the responses measured by the hindfoot drop test or the lateral hop test. A general decline in performance in the tail tip curl test was noted in male rats exposed to Durad MP280. Results of this test in female rats were erratic. The loss of test subjects toward the termination of the exposure greatly hinders a conclusive interpretation.

Hematology values of rats at exposure termination are shown in Table 58. All values noted were well within normal biological species limits. Leukocytosis was noted in male rats exposed to 100 mg/m^3 Durad MP280. Its relationship to exposure is unclear. Female rats exposed to Fyrquel 220 or Durad MP280 at 100 mg/m^3 had lower mean RBC counts at termination. This was not noted in female rats exposed to the lowest concentration of these two materials, nor in female rats exposed to Houghto-Safe 273.

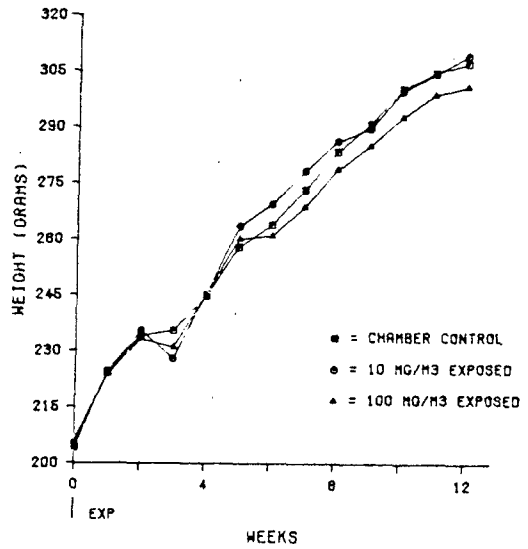


Figure 22. Effect of Houghto-Safe 273 exposure on male rat body weight.

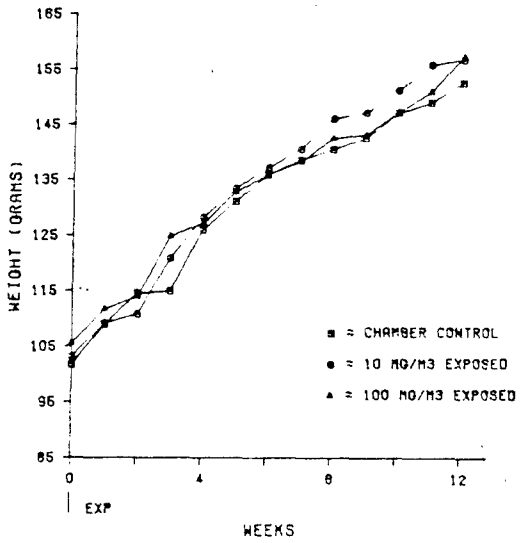


Figure 23. Effect of Houghto-Safe 273 exposure on male hamster body weight.

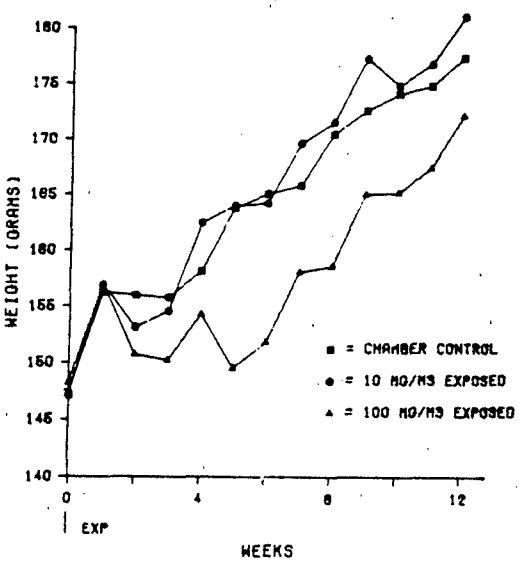


Figure 24. Effect of Houghto-Safe 273 exposure on female rat body weight.

Results of clinical chemistry and blood cholinesterase measurements are not available from NMRI/TD.

A number of values obtained for the heart, spleen, and brain weights of animals exposed to the three hydraulic fluids were statistically different from control values. No remarkable trends were noted in any of these values, however. Notable increases in kidney weight were observed in the male rats exposed to Fyrquel 220 and Durad MP280 at 100 mg/m^3 (Table 59). Kidney weights of the male rats exposed to the lower concentrations of these materials were comparable to controls. Significant elevations in liver weight occurred in male rats exposed to Fyrquel 220 or Durad MP280. Relative liver weight of male rats exposed to 100 mg/m^3 was increased more than 60% and 40% for Fyrquel 220 and Durad MP280, respectively. Exposure to 10 mg/m^3 of these materials produced less dramatic increases (13% Fyrquel 220; 4% Durad MP280). The relative liver weight of the male rats exposed to 10 mg/m^3 Fyrquel 220 was statistically different ($p \leq 0.01$) from the controls, however.

TABLE 57. MEAN SCORES OF CNS EXAMINATION OF RATS EXPOSED FOR 90 DAYS TO HYDRAULIC FLUIDS[a]

	MALE			
	2 Weeks	4 Weeks	8 Weeks	Termination
Tail Tip Curl				
Control	2	2	2	2
Fyrquel 220	2	2	2	1.6
Durad MP280	2	2	1.4	0.8
Hind Foot Drop				
Control	0	0	0	0
Fyrquel 220	0	0	0	0
Durad MP280	0	0	0	0
Lateral Hop				
Control	1	1	1	1
Fyrquel 220	1	1	1	0.8
Durad MP280	1	1	0.6	0.8

	FEMALE			
	2 Weeks	4 Weeks	8 Weeks	Termination
Tail Tip Curl				
Control	1.8	1.2	1.5[b]	1.5[b]
Fyrquel 220	1.8	1.2	1	2
Durad MP280	1.4	1.8	1[c]	1.5[d]
Hind Foot Drop				
Control	0	0	0[b]	0[b]
Fyrquel 220	0	0	0	0.2
Durad MP280	0	0	0[c]	0[d]
Lateral Hop				
Control	1	1	1[b]	1[b]
Fyrquel 220	0.6	1	1	1
Durad MP280	1	1	0.7[c]	1[d]

[a]Mean value, N=5, units described in test
[b]N = 4
[c]N = 3
[d]N = 2

TABLE 58. HEMATOLOGY VALUES OF RATS EXPOSED TO TRIARYLPHOSPHATE OR POLYGLYCOL HYDRAULIC FLUIDS[a]

MALE RATS

	Control (N = 8)	Fyrquel 220 10 mg/m³ (N = 8)	Fyrquel 220 100 mg/m³ (N = 10)	Durad MP280 10 mg/m³ (N = 10)	Durad MP280 100 mg/m³ (N = 10)	Houghto-Safe 273 10 mg/m³ (N = 8)	Houghto-Safe 273 100 mg/m³ (N = 8)
RBC (10^6 cells/m³)	8.22 ± 0.04	8.09 ± 0.1	8.04 ± 0.1	8.08 ± 0.1	4.19 ± 0.1	7.85 ± 0.1[b]	8.14 ± 0.1
WBC (10^3 cells/m³)	5.2 ± 0.3	5.4 ± 0.2	5.5 ± 0.2	5.5 ± 0.4	7.4 ± 0.4[b]	4.6 ± 0.24	5.8 ± 0.2
HCT (%)	41.8 ± 0.4	40.9 ± 0.6	41.3 ± 0.8	40.1 ± 0.5[c]	40.8 ± 0.4	40.1 ± 0.7[c]	43.4 ± 0.6
HGB (g/dl)	14.6 ± 0.2	14.3 ± 0.1	14.6 ± 0.2	14.4 ± 0.2	14.6 ± 0.2	13.9 ± 0.2[c]	14.7 ± 0.2
MCV (cu microns)	51.0 ± 0.4	50.7 ± 0.3	51.3 ± 0.2	49.6 ± 0.3[b]	50.0 ± 0.3[c]	51.0 ± 0.2	52.0 ± 0.4[c]
MCH (micromicrograms)	17.8 ± 0.2	17.7 ± 0.1	18.2 ± 0.1	17.9 ± 0.2	17.9 ± 0.1	17.7 ± 0.1	17.6 ± 0.1
MCHC (%)	34.9 ± 0.1	34.9 ± 0.3	35.4 ± 0.3	35.9 ± .4[c]	35.8 ± 0.3[c]	34.7 ± 0.3	33.8 ± 0.2[c]

FEMALE RATS

	Control (N = 8)	Fyrquel 220 10 mg/m³ (N = 10)	Fyrquel 220 100 mg/m³ (N = 10)	Durad MP280 10 mg/m³ (N = 10)	Durad MP280 100 mg/m³ (N = 5)	Houghto-Safe 273 10 mg/m³ (N = 10)	Houghto-Safe 273 100 mg/m³ (N = 10)
RBC (10^6 cells/m³)	7.72 ± 0.07	8.00 ± 0.2	7.45 ± 0.1[c]	7.70 ± 0.1	7.43 ± 0.1	8.01 ± 0.1	8.02 ± 0.1[b]
WBC (10^3 cells/m³)	3.6 ± 0.2	4.3 ± 0.2	3.5 ± 0.2	4.0 ± 0.3	4.2 ± 0.2	4.6 ± 0.4[c]	4.4 ± 0.3
HCT (%)	42.0 ± 0.5	42.7 ± 0.8	38.9 ± 0.5[b]	47.3 ± 1.9[c]	38.8 ± 0.3[b]	43.5 ± 0.7	43.1 ± 0.3[b]
HGB (g/dl)	14.6 ± 0.1	14.8 ± 0.3	13.7 ± 0.2[b]	14.1 ± 0.1	13.4 ± 0.2[b]	14.8 ± 0.2	14.7 ± 0.1
MCV (cu microns)	54.3 ± 0.4	53.4 ± 0.2	53.5 ± 0.3	61.4 ± 2.4[c]	52.4 ± 0.3[c]	54.3 ± 0.2	53.8 ± 0.3
MCH (micromicrograms)	18.9 ± 0.1	18.6 ± 0.1[c]	18.3 ± 0.1[b]	18.3 ± 0.1[b]	18.0 ± .03[b]	18.5 ± 0.1[c]	18.4 ± 0.1[b]
MCHC (%)	34.7 ± 0.3	34.8 ± 0.2	34.3 ± 0.2	30.2 ± 1.2[b]	34.3 ± 0.2	34.1 ± 0.1[c]	34.2 ± 0.1

[a]Mean ± S.E.
[b]Statistically different from control values, $p \leq 0.01$.
[c]Statistically different from control values, $p \leq 0.05$.

TABLE 59. ORGAN WEIGHT OF MALE RATS EXPOSED TO TRIARYLPHOSPHATE OR POLYGLYCOL HYDRAULIC FLUIDS[a]

	Control	Fyrquel 220 10 mg/m³	Fyrquel 220 100 mg/m³	Durad MP280 10 mg/m³	Durad MP280 100 mg/m³	Houghto-Safe 273 10 mg/m³	Houghto-Safe 273 100 mg/m³
Body Wt, g	288 ± 3	294 ± 4	298 ± 4	305 ± 4	277 ± 4[b]	303 ± 16	294 ± 14
Heart Wt, g	0.88 ± 0.02	0.87 ± 0.02	0.88 ± 0.02	0.86 ± 0.02	0.80 ± 0.02[c]	0.85 ± 0.0_	0.86 ± .02
Heart/100 g Body Wt	0.29 ± 0.01	0.29 ± 0.01	0.30 ± 0.01	0.28 ± 0.004	0.29 ± 0.01	0.28 ± 0.004	0.29 ± 0.01
Spleen Wt, g	0.56 ± 0.01	0.60 ± 0.01[b]	0.55 ± 0.01	0.61 ± 0.01[b]	0.57 ± 0.01	0.61 ± 0.01[b]	0.58 ± 0.01[c]
Spleen/100 g Body Wt	0.19 ± 0.002	0.20 ± 0.01[b]	0.19 ± 0.002	0.20 ± 0.002[b]	0.21 ± 0.004[b]	0.20 ± 0.004[b]	0.20 ± 0.004[b]
Kidney Wt, g	2.00 ± 0.21	2.05 ± 0.04	2.25 ± 0.03[b]	2.05 ± 0.04	2.04 ± 0.04	2.11 ± 0.04[c]	1.95 ± .02
Kidney/100 g Body Wt	0.67 ± 0.005	0.69 ± 0.01	0.75 ± 0.01[b]	0.67 ± 0.01	0.74 ± 0.01[b]	0.69 ± 0.01[c]	0.66 ± 0.01
Brain Wt, g	1.84 ± 0.01	1.83 ± 0.01	1.82 ± 0.02	1.81 ± 0.02	1.82 ± 0.02	1.86 ± 0.02	1.85 ± 0.01
Brain/100 g Body Wt	0.62 ± 0.005	0.62 ± 0.01	0.61 ± 0.01	0.59 ± 0.01[b]	0.66 ± 0.02[b]	0.62 ± 0.01	0.63 ± 0.01
Liver Wt, g	7.89 ± 0.18	8.77 ± 0.18[b]	12.93 ± 0.23[b]	8.38 ± 0.17	10.00 ± 0.29[b]	8.22 ± 0.26	7.55 ± 0.17
Liver/100 g Body Wt	2.64 ± 0.05	2.97 ± 0.06[b]	4.35 ± .07[b]	2.75 ± 0.03	3.60 ± 0.06[b]	2.70 ± 0.06	2.57 ± 0.04

[a]Mean ± S.E. of 19 or 20 observations per group.
[b]Statistically different from control values $p \leq 0.01$.
[c]Statistically different from control values, $p \leq 0.05$.

Analysis of female rat organ weight indicates results similar to those obtained in male rats, i.e., elevated kidney and liver weights in animals exposed to Fyrquel 220 and Durad MP280 (Table 60). Increases in relative liver weights were comparable between male and female rats. An approximate 10% increase occurred in

females exposed to 10 mg/m^3 of either material, while increases of 70% and 45% occurred in females exposed to Fyrquel 220 or Durad MP280, respectively, at the high concentration.

TABLE 60. ORGAN WEIGHT OF FEMALE RATS EXPOSED TO TRIARYLPHOSPHATE OR POLYGLYCOL HYDRAULIC FLUIDS[a]

	Control	Fyrquel 220		Durad MP280		Houghto-Safe 273	
		10 mg/m^3	100 mg/m^3	10 mg/m^3	100 mg/m^3	10 mg/m^3	100 mg/m^3
Body Wt, g	168 ± 3	165 ± 2	157 ± 1[b]	165 ± 2	151 ± 4[b]	174 ± 2	162 ± 2
Heart Wt, g	0.60 ± 0.01	0.58 ± 0.01	0.57 ± 0.01	0.57 ± 0.01	0.52 ± 0.02[b]	0.60 ± 0.01	0.57 ± 0.01
Heart/100 g Body Wt	0.36 ± 0.01	0.35 ± 0.01	0.37 ± 0.01	0.35 ± 0.01	0.35 ± 0.01	0.35 ± 0.01	0.35 ± 0.01
Spleen Wt, g	0.37 ± 0.01	0.38 ± 0.01	0.36 ± 0.01	0.40 ± 0.01[c]	0.38 ± 0.01	0.38 ± 0.01	0.38 ± 0.0.
Spleen/100 g Body Wt	0.22 ± 0.002	0.23 ± 0.004[c]	0.23 ± 0.004	0.24 ± 0.004[b]	0.25 ± 0.01[b]	0.22 ± 0.004	0.23 ± 0.004
Kidney Wt, g	1.22 ± 0.02	1.29 ± 0.0-[b]	1.37 ± 0.02[b]	1.26 ± 0.02	1.29 ± 0.04	1.29 ± 0.01[b]	1.22 ± 0.01
Kidney/100 g Body Wt	0.73 ± 0.01	0.78 ± 0.01[b]	0.87 ± 0.02[b]	0.76 ± 0.01[c]	0.85 ± 0.01[b]	0.74 ± 0.01	0.75 ± 0.01
Brain Wt, g	1.71 ± 0.01	1.73 ± 0.01	1.70 ± 0.01	1.69 ± 0.01	1.71 ± 0.01	1.70 ± 0.02	1.72 ± 0.01
Brain/100 g Body Wt	1.02 ± 0.02	1.06 ± 0.02	1.09 ± 0.02[b]	1.03 ± 0.01	1.14 ± 0.02[b]	0.98 ± 0.02	1.06 ± 0.01
Liver Wt, g	4.20 ± 0.08	4.48 ± 0.10[b]	4.67 ± 0.11[b]	4.63 ± 0.12[b]	5.44 ± 0.16[b]	4.71 ± 0.08[b]	4.20 ± 0.08
Liver/100 g Body Wt	2.51 ± 0.04	2.83 ± 0.04[b]	4.28 ± 0.06[b]	2.80 ± 0.04[c]	3.63 ± 0.11[b]	2.72 ± 0.04[b]	2.59 ± 0.04

[a]Mean ± S.E. of 15 or 20 observations per group.
[b]Statistically different from control values, $p < 0.01$.
[c]Statistically different from control values, $p < 0.05$.

Organ weights of male and female rats exposed to Houghto-Safe 273 failed to indicate any striking effects. Liver weights of females exposed to 10 mg/m^3 Houghto-Safe 273 was increased over controls, but the absence of a dose response suggests that this finding was unrelated to exposure.

In a previously reported stuay, increased liver weights were noted in male and female rats exposed to 250 mg/m^3 Fyrquel 220 or Durad MP280 intermittently for 21 days. Kidney weights were not significantly altered during those exposures.

Gross pathologic findings on rabbits and hamsters at the conclusion of the exposure period showed no remarkable lesions. Enlargement of the adrenals was noted in the female rats from the Durad MP280 groups as well as the high level Fyrquel 220 group. This was a subjective judgment during necropsy which was not verified by organ weight. This lesion was seen in male rats from the high level Durad MP280 group only (Tables 61 and 62). The other lesions of note at termination of exposure were testicular atrophy in 25% of the Durad MP280 100 mg/m^3 rat group and petechial hemorrhage in 50% of the male rats exposed to 100 mg/m^3 Houghto-Safe 273.

TABLE 61. GROSS PATHOLOGIC LESIONS[a] SEEN IN MALE RATS IMMEDIATELY FOLLOWING 90 DAY AEROSOL EXPOSURE TO DURAD MP280, FYRQUEL 220, AND HOUGHTO-SAFE 273

		Fyrquel 220 (mg/m^3)		Durad MP280 (mg/m^3)		Houghto-Safe 273 (mg/m^3)	
Gross Lesion	Controls	10	100	10	100	10	100
Lungs:							
Spots or hemorrhage	2/19	3/20	2/20	1/20	1/20	1/20	10/20
Liver:							
Nodules	0/19	0/20	2/20	0/20	0/20	2/20	1/20
Adrenals:							
Enlargement	0/19	0/20	0/20	0/20	6/20	0/20	0/20
Reproductive:							
Testicular atrophy	0/19	0/20	0/20	0/20	5/20	0/20	0/20

[a]Number of lesions/number of animals examined

Results of the blood cholinesterase, clinical chemistry, and histopathologic examination are incomplete. However, based on the information to date, it appears that Durad MP280 is the more toxic of the two triarylphosphate compounds based on the gross pathology and mortality. Body weights of all species were suppressed during exposure to 100 mg/m^3 of Durad MP280.

Houghto-Safe 273 appears to be the least toxic of the three hydraulic fluids. Final conclusions on the toxicity of the three hydraulic fluids will be made after receipt of the outstanding data.

TABLE 62. GROSS PATHOLOGIC LESIONS[a] SEEN IN FEMALE RATS IMMEDIATELY FOLLOWING 90 DAY AEROSOL EXPOSURE TO DURAD MP280, FYRQUEL 220, AND HOUGHTO-SAFE 273

Gross Lesion	Controls	Fyrquel 220 (mg/m^3)		Durad MP280 (mg/m^3)		Houghto-Safe 273 (mg/m^3)	
		10	100	10	100	10	100
Lungs:							
Spots or hemorrhage	1/18	1/20	0/19	1/20	1/15	1/20	3/19
Liver:							
Nodules	1/18	1/20	0/19	0/20	0/15	1/20	0/19
Adrenals:							
Enlargement	0/18	1/20	7/19	5/20	8/15	0/20	1/19
Reproductive:							
Ovarian cysts	2/18	1/20	0/19	2/20	2/15	2/20	0/19
Enlarged or dark ovaries	0/18	0/20	1/19	1/20	0/15	0/20	0/19
Uterine horn (with fluid)	3/18	2/20	3/19	3/20	1/15	5/20	2/19

[a]Number of lesions/number of animals examined

ACUTE DELAYED NEUROTOXICITY TESTING OF MIXTURES OF ORGANOPHOSPHATE ESTER BASED HYDRAULIC FLUIDS FROM SHIP SYSTEMS

A hydraulic fluid meeting military specification MIL-H-19457B was replaced by one meeting MIL-H-19457C (SH) in the hydraulic systems of a U. S. Naval ship. The major difference between the specifications is that the former requires a formulation of phosphoric esters, while MIL-H-19457C (SH) requires the material to be tri-tertiarybutylphenyl phosphate based. After changeover there was concern that residual MIL-H-19457B material in the four ship hydraulic systems had contaminated the replacement fluid. Analysis of TOCP content showed that the degree of contamination differed in the four hydraulic systems. Samples were removed from each of the four hydraulic fluid system tanks for determination of degree of contamination.

NMRI/TD requested that an acute delayed neurotoxicity study be performed to determine if the level of contamination had created a hazard. Two of the four samples were tested; the sample with the highest TOCP concentration and the sample having the lowest TOCP concentration. A vehicle control and a TOCP positive control were tested concurrently.

The hydraulic fluids and the positive control TOCP were administered to unfasted hens as solutions in corn oil. Gastric intubation was accomplished employing a syringe fitted with a 6-inch infant catheter. The injection volume for the hens was 100 ml/kg which resulted in the average hen receiving a volume of 1.3 ml. The hens were weighed individually to determine the proper dose volume.

The following dosing regimen was performed on five consecutive days:

Hydraulic Fluid - Elevator #4; NMRI/TD 2268-3 (High TOCP)
Groups of four hens treated with each of the following doses: 240, 300, 360, 420 mg/kg/day.

Hydraulic Fluid - Elevator #1; NMRI/TD 2268-1 (Low TOCP)
Groups of four hens treated with each of the following doses: 240, 300, 360, 420 mg/kg/day.

TOCP
Groups of four hens treated with each of the following doses: 60, 75, 90 mg/kg/day.

Corn Oil
Twelve hens given 1 ml/kg/day.

Positive neurotoxic symptoms were observed in all hens given TOCP. The resultant scores 21 days after the initial peroral dose are shown in Table 63. No neurotoxic signs were seen in hens administered corn oil or either hydraulic fluid sample.

On the basis of these experiments, the hydraulic fluid mixtures should represent no neurotoxic hazard to personnel.

TABLE 63. SCORING OF NEUROTOXIC EFFECTS OBSERVED 21 DAYS FOLLOWING THE FIRST PERORAL DOSE OF TOCP

Dose Level (mg/kg)	Group Score
90	40
75	39
60	33

THE TOXICOLOGIC EVALUATION OF POLYALPHAOLEFIN HYDRAULIC FLUIDS

The THRU was requested to evaluate the acute toxicity of six synthetic hydraulic fluids developed by the Naval Research and Development Center. These polyalphaolefin base materials are intended for replacement of the petroleum oil-based hydraulic fluids described by Military Specifications; MIL-H-17672C, MIL-L-17331G, and MIL-F-17111A.

Each of the synthetic hydraulic fluids contain polyalphaolefins as the major ingredient and minor components including antioxidant, antiwear additive, and rubber swell additive. The exact composition of the materials remains proprietary information. A list of the materials and the various codes is as follows:

DTNSRDC No.[a]	NMRI/TD No.	Supplier
N448[b]	1160-8	Gulf R & D Company
N501	1160-4	Gulf R & D Company
N517	1160-1	American Oil & Supply Company
N518	1160-3	American Oil & Supply Company
N525	1160-6	Royal Lubricants, Incorporated
N527	1160-5	Bray Oil Company

[a]David Taylor Naval Ship Research and Development Center
[b]This material is polyalphaolefin base stock of sample #N501.

Samples of the six fluids were supplied by the Naval Medical Research Institute/Toxicology Detachment, Wright-Patterson Air Force Base, Ohio.

The most significant exposure routes for hydraulic fluids are expected to be dermal, due to leaks or spills, and aerosol inhalation from pressurized system leaks. This study reflects these

potential routes of exposure and included tests for eye and skin irritation, skin sensitization, single dose oral, and dermal toxicity and aerosol inhalation.

Animals

Male albino, Hartley guinea pigs, weighing between 300 and 500 grams, male and female New Zealand albino rabbits weighing approximately 2.3 kgs, and male and female Sprague-Dawley rats (200-300g and 150-250g, respectively) were used in these studies. The guinea pigs were supplied by Murphy Breeding Labs, Plainfield, Indiana and the rabbits were supplied by Plummer Rabbit Ranch, Peebles, Ohio. Rats were supplied by Charles River Breeding Labs, Wilmington, Massachusetts. Food and water were provided ad libitum.

Primary Skin Irritation

A patch-test method was conducted to measure the degree of primary dermal irritation of intact and abraded skin of albino rabbits.

Six rabbits were clipped of all possible hair on the back and flanks 24 hours prior to exposure to allow for recovery of the skin from any abrasion resulting from the clipping. Two areas in the back, one on each side, were designated as patch-test areas. One area was abraded by making minor incisions through the stratum corneum. These abrasions were not sufficiently deep to disturb the derma or to produce bleeding.

The hydraulic fluid was applied as 0.5 ml to the designated patch-test area and was covered by a one-inch square of surgical gauze two single layers thick. The gauze patches were held in place with strips of elastoplast tape. The entire area was covered with a rubber dental dam strip and secured with more elastoplast tape. The patches remained in place for 24 hours. During that time, the rabbits were fitted with leather restraining collars to prevent disturbance of the patch area while allowing freedom of movement and access to food and water.

After 24 hours, the wrap and patches were carefully removed, and the test areas were evaluated for irritation using the Draize (1959) table as a reference standard. Readings were also made at 72 hours (48 hours after the first reading). The total score of both readings for all six rabbits was divided by 24 to yield a primary irritation score.

Primary Eye Irritation

A 0.1 ml sample of hydraulic fluid was applied to one eye of each of nine albino rabbits. The opposite eye was untreated and served as a control. The treated eyes of three rabbits were flushed with lukewarm water approximately 30 seconds after instillation of the fluid. Examinations for gross signs of eye irritation were made at scheduled observation periods following application. Scoring of the irritative effects was according to the method of Draize (1959). In this scoring system, injuries to the cornea and iris may represent as much as 80% of the total score because of their essential roles in vision.

Skin Sensitization

Ten male albino guinea pigs, Hartley strain, six to eight weeks of age, were used for each hydraulic fluid. An area on the back of each animal directly above the forelegs was clipped with electric clippers and the fur chemically removed with a commercial depilatory on the morning of the first insult exposure as recommended by Maguire (1973). Test solutions, 0.1 ml at each application, were applied to this area on a 1/2 x 1/2 inch cotton gauze square, covered with dental dam, and held in place with adhesive tape. The first insult patch was allowed to remain in place for two days, then removed, and a second application of 0.1 ml was made. Two days later, this patch was removed, a total of 0.2 ml of Freund's adjuvant per animal injected intradermally, using 2 or 3 points adjacent to the insult site, and then a new patch containing 0.1 ml of the test material applied. Three days after the third application, a fresh patch of 0.1 ml of the material was applied. The last patch was removed two days later, then the animals were allowed to rest for two weeks. Each time the insult patches were removed, the condition of the skin at the application site was evaluated and recorded. When the last patch was removed, the toes on the hind feet of the guinea pigs were taped to prevent the animal from scratching the irritated area.

After the two-week rest period, the right flanks of the same animal were clipped and challenged with the test solution. The challenge application was not occluded. The skin response at these sites was recorded at 24 and 48 hours after application according to the evaluation method of Draize (1959) and shown in Table 64. Any animal eliciting a score of 2 or more at the test solution challenge site would be rated as a positive responder.

TABLE 64. GRADING OF SKIN REACTIONS IN THE MAGUIRE GUINEA PIG SENSITIZATION TEST

Erythema	Edema
0 - None	0 - None
1 - Very slight pink	1 - Very slight
2 - Slight pink	2 - Slight
3 - Moderate red	3 - Moderate
4 - Very Red	4 - Marked

Acute Oral

Syringes equipped with special oral dosing needles were used to administer the materials to the rats. Rats used for oral dosing studies were fasted 12 hours prior to dosing.

Testing was initiated by dosing 5 rats of each sex at a concentration of 5 ml/kg body weight. This concentration was used as an upper limit cut off level. If no toxicity was evident during a 14-day observation period, no further testing was conducted. Mortality was recorded for 14 days after dosing. A 14 day LD_{50} with 90% confidence limits was calculated using the probit analysis method of Finney (1971).

Acute Dermal Toxicity

All rabbits were clipped as closely as possible with an Oster clipper having surgical blades and vacuum attachment. The backs of the rabbits and the sides down to about half way to the stomach area were clipped from the shoulders to the top of the rear leg area.

The materials were applied in equal amounts to both sides of the rabbit's back and remained in contact with the skin for 24 hours. The dose was kept in place by applying 4" x 4" 8 ply gauze patches over the compound on each side of the back. Latex rubber dental dam was then applied over the entire clipped area and elastoplast tape used to wrap the entire midsection of the rabbit, keeping the dose in place. Specially designed restraining harnesses were fitted to each rabbit at the time of dosing and kept in place during the entire dosing period. These harnesses prevented excessive movement of the rabbits and prevented them from chewing on the taped area. The harnesses, however, allowed the rabbits to eat and drink

during the dosing period. Upon removal of the wrapping, the skin of the rabbit was wiped (not washed) in order to remove excess test material.

Testing was initiated by dosing 5 rabbits of each sex at an upper limit cut off concentration of 2 ml/kg body weight. If no toxicity was evident during the 14 day observation period, no further testing was conducted.

Acute Inhalation

The fluids were aerosolized into a Plexiglas® 60-liter cubic chamber. Multi-jet Collison Nebulizers in a 250 ml three neck round bottom flask were used to generate the aerosol. Nominal concentrations were obtained by material balance calculations.

The aerosol concentration was analyzed using an Anderson Cascade Impactor. The analysis was gravimetric with the individual stages weighed before and after sampling. Particle sizing with the same instrument was done twice for each exposure with the results averaged. From these data, the aerodynamic mass median diameter and standard geometric mean were determined.

Testing was initiated by exposing five rats of each sex to a mass concentration of 5 mg/L. If no toxicity was evident during the 14 day observation period, no further testing was conducted. When mortality occurred, further testing was conducted using 10 rats per sex at each concentration level. The 14 day LC_{50} with 95% confidence limits was calculated using the probit analysis method of Finney (1971).

Results

Primary Skin Irritation: A patch-test method was conducted to measure the degree of primary dermal irritation of intact and abraded skin of 6 albino rabbits. Readings of the skin reactions were evaluated by 2 independent graders using the Draize table as a reference standard. The readings were recorded at 24 and 72 hours after treatment.

Two of the six fluids, when applied to intact and abraded rabbit skin produced mild irritation responses (Table 65). The response of the abraded skin was no greater than that of the intact skin. The remaining four fluids did not produce a primary irritation response on rabbit skin.

TABLE 65. RABBIT PRIMARY SKIN IRRITATION RESULTS

DTNSRDC No.	Primary Irritation Index[a]	Effect
N448	<0.05	Nonirritating
N501	1.29	Mild Irritant
N517	0.75	Mild Irritant
N518	0	Nonirritating
N525	0	Nonirritating
N527	0	Nonirritating

[a]Primary Irritation Index = $\frac{\text{Total Reaction Score}}{\text{No. of Rabbits x Test Sites per Rabbit x No. of Readings}}$

Primary Eye Irritation: A 0.1 ml sample of hydraulic fluid was applied to one eye of each of nine albino rabbits. The opposite eye was untreated and served as a control. The treated eye of three rabbits was flushed with lukewarm water approximately 30 seconds after instillation of the fluid. Examinations for gross signs of eye irritation were made at scheduled observation periods following application and the scoring of the irritative effects was done according to the method of Draize.

None of the six hydraulic fluids caused any ocular irritation in the rabbits. No differences could be noticed when comparing the eyes treated with test material and either the treated and washed eyes or the respective untreated control eyes at the scheduled observation periods.

Skin Sensitization: Guinea pigs were tested for skin sensitization response using the Maguire guinea pig sensitization test. One fluid, sample N501, produced mild irritation of guinea pig skin when used undiluted or as a 50% mixture in mineral oil and was therefore tested as a 25% solution in mineral oil while the remaining fluids were tested undiluted.

None of the hydraulic fluid samples tested by the Maguire method caused a dermal reaction in guinea pigs. The responses to the challenge application of the hydraulic fluids to ten guinea pigs following a two week incubation period were negative in all six cases.

Oral Toxicity: Five male and five female rats received doses of 5 ml/kg and were held 14-days for evaluation of toxicity. These dose levels failed to produce signs of toxic stress and no deaths occurred. All groups exhibited normal weight gains during the 14 day observation period.

Dermal Toxicity: No mortality resulted from dermal exposure to 2 ml/kg of the fluids. Some of the treated rabbits exhibited lethargy during the 24 hour dosing period but the lethargy was not noted after removal of the dose patches and collars. All groups showed consistent weight gains during the 14 day postexposure holding period.

Inhalation Toxicity: Five male and five female Sprague-Dawley rats were exposed to aerosol concentrations in excess of 5 mg/L and held for a 14 day observation period.

Inhalation exposure to N448, N517, N518, N525, or N527 in aerosol concentrations shown in Table 67 failed to produce mortality and no overt toxic signs were noted in any of the rats exposed to these materials. Body weights are shown in Table 66. Slight transient weight losses were noted in some of the rats; however, a general weight gain during the 14 day observation period was observed.

Exposure to an aerosol concentration of 6.43 mg/L N501 killed all 5 male rats and 4 of 5 female rats within 24 hours. Signs of toxicity noted in the rats exposed to N501 included lethargy and labored respiration.

Since no mortality occurred in the rats exposed to N448, N517, N518, N525, or N527 at concentrations in excess of 5 mg/L, no further testing was conducted. Further exposures to N501 were conducted to establish an LC_{50} with 95% confidence limits. The exposure concentration and mortality ratios are shown in Table 68.

Death resulting after exposure to the aerosolized N501 occurred within hours. Signs of toxicity included rough coat, labored breathing, and lethargy. Mean body weights of the surviving rats from each exposure group are shown in Table 69. Transient weight loss was evident in all groups but was reversed in most cases by the 4th postexposure day. Severity and duration of weight loss was dose related.

TABLE 66. EFFECT OF 4-HOUR INHALATION EXPOSURE TO SYNTHETIC HYDRAULIC FLUID AEROSOL ON RAT BODY WEIGHT[a] (gms)

Male Rats

Day	Material N448	N501	N517	N518	N525	N527
0	250 ± 8	247 ± 2	228 ± 3	281 ± 4	294 ± 10	291 ± 5
1	261 ± 5	----	274 ± 2	298 ± 5	300 ± 12	293 ± 5
2	264 ± 5	----	298 ± 3	298 ± 4	308 ± 11	294 ± 5
4	280 ± 7	----	305 ± 3	304 ± 6	308 ± 11	290 ± 5
7	297 ± 6	----	310 ± 3	318 ± 6	330 ± 12	308 ± 5
10	304 ± 3	----	326 ± 3	333 ± 6	349 ± 13	324 ± 6
14	324 ± 9	----	335 ± 3	346 ± 7	358 ± 12	335 ± 5

Female Rats

Day	Material N448	N501	N517	N518	N525	N527
0	183 ± 7	172 ± 6	196 ± 4	188 ± 7	195 ± 7	109 ± 6
1	194 ± 7	163[b]	184 ± 7	203 ± 6	196 ± 8	198 ± 5
2	194 ± 7	171	203 ± 9	200 ± 7	193 ± 6	196 ± 5
4	203 ± 8	176	201 ± 9	200 ± 6	198 ± 7	201 ± 5
7	211 ± 8	190	205 ± 8	210 ± 7	205 ± 8	207 ± 6
10	214 ± 10	201	213 ± 11	214 ± 8	211 ± 8	211 ± 6
14	223 ± 10	203	220 ± 10	223 ± 9	217 ± 8	220 ± 6

[a]Mean ± S.E., N=5
[b]N=1

TABLE 67. CONCENTRATION AND PARTICLE SIZE MEASUREMENTS OF THE SYNTHETIC HYDRAULIC FLUIDS DURING INHALATION EXPOSURES

Material	Concentration Analyzed (mg/L)	Concentration Nominal (mg/L)	Particle Size MMAD (μm)	Particle Size σg
N448	10.72 ± 0.44	11.47	1.77	2.08
N501	6.43 ± 0.81	8.42	2.84	1.85
N517	5.43 ± 0.52	7.82	1.85	2.02
N518	5.35 ± 0.11	8.19	1.83	2.55
N525	5.33 ± 0.22	7.36	2.00	2.10
N527	5.47 ± 0.30	7.77	1.7	2.11

TABLE 68. 4-HOUR INHALATION LC_{50} VALUES OF RATS EXPOSED TO POLYALPHAOLEFIN MATERIAL N501

Male Rats

Particle Size

Concentration (mg/L) Analyzed	Nominal	MMAD (μm)	σg	Mortality No. Dead/No. Exposed
5.03 ± 0.07	7.62	2.81	1.85	9/10
3.02 ± 0.16	4.71	3.40	1.65	8/10
2.51 ± 0.12	3.23	3.08	1.54	5/10
2.02 ± 0.07	3.11	3.85	1.58	4/10
1.02 ± 0.04	1.43	3.77	1.68	0/10

LC_{50} and (95% C.L.) = 2.39 (1.87 to 2.93) mg/L

Female Rats

Particle Size

Concentration (mg/L) Analyzed	Nominal	MMAD (μm)	σg	Mortality No. Dead/No. Exposed
5.04 ± 0.11	7.03	2.93	1.68	10/10
4.01 ± 0.14	5.73	2.99	1.66	9/10
3.03 ± 0.18	4.02	3.46	1.65	7/10
2.05 ± 0.16	2.88	2.83	1.53	6/10
1.06 ± 0.07	2.01	3.27	1.97	3/10

LC_{50} and (95% C.L.) = 1.67 (0.95 to 2.23) mg/L

Discussion

Results of the acute tests conducted on the six hydraulic fluids are summarized in Table 70. Of the six fluids tested, only N501 demonstrated any significant acute toxicity. It was irritating to both rabbit and guinea pig skin and had a four-hour aerosol inhalation LC_{50} value of approximately 2 mg/L for both male and female rats.

TABLE 69. MEAN[a] BODY WEIGHT (GMS) OF RATS EXPOSED FOR 4 HOURS TO POLYALPHAOLEFIN MATERIAL N501

Male Rats

Concentration (mg/L)

Day	5.03	N	3.02	N	2.51	N	2.02	N	1.02	N
0	228 ± 3	10	233 ± 4	10	262 ± 3	10	239 ± 4	10	256 ± 4	10
1	185	1	204 ± 7	2	N/A	--	220 ± 6	6	251 ± 5	10
2	185	1	207 ± 2	2	249 ± 6	5	225 ± 10	6	264 ± 5	10
4	212	1	226 ± 9	2	265 ± 4	5	242 ± 9	6	266 ± 5	10
7	236	1	244 ± 14	2	283 ± 4	5	265 ± 9	6	294 ± 6	10
10	258	1	265 ± 19	2	305 ± 5	5	286 ± 8	6	316 ± 7	10
14	288	1	290 ± 20	2	331 ± 6	5	316 ± 9	6	329 ± 8	10

Female Rats

Concentration (mg/L)

Day	5.04	N	4.01	N	3.03	N	2.05	N	1.06	N
0	175 ± 3	10	181 ± 3	10	225 ± 3	10	186 ± 5	10	209 ± 5	10
1	---	0	158 ± 2	2	204 ± 4	4	170 ± 11	4	200 ± 8	8
2	---	0	153	1	205 ± 6	3	182 ± 11	4	207 ± 7	7
4	---	0	165	1	216 ± 7	3	188 ± 10	4	213 ± 7	7
7	---	0	185	1	237 ± 10	3	203 ± 12	4	219 ± 7	7
10	---	0	200	1	247 ± 14	3	218 ± 14	4	233 ± 8	7
14	---	0	217	1	246 ± 9	3	225 ± 7	4	233 ± 8	7

[a]Mean ± S.E.

TABLE 70. SUMMARY OF ACUTE TEST RESULTS FOR SYNTHETIC HYDRAULIC FLUIDS

DTNSRDC Number	Eye Irritation	Skin Irritation	Sensitization	Oral LD_{50} (mg/kg)	Dermal LD_{50} (ml/kg)	Inhalation LC_{50} (95% C.L.) (mg/L)
N448	Negative	Negative	Negative	>5	>2	1[b]
N501	Negative	Mild	Negative[a]	>5	>2	2.39 (1.87-2.93)[c] 1.67 (0.95-2.23)[d]
N517	Negative	Mild	Negative	>5	>2	>[b]
N518	Negative	Negative	Negative	>5	>2	>[b]
N525	Negative	Negative	Negative	>5	>2	>[b]
N527	Negative	Negative	Negative	>5	>2	>[b]

[a]Tested as a 25% solution in mineral oil
[b]No deaths at 5 mg/L
[c]Male rat
[d]Female rat

In the absence of histopathologic examination at this time, it is not possible to comment on the irritation aspects of N501 in the respiratory system. The base polyalphaolefin for N501 is the fluid numbered N448. N448 failed to produce any signs of skin irritation

or sensitization and was not lethal at the upper limit concentrations tested. This suggests that the toxic signs noted in the acute tests of N501 were caused by one or more of the additives.

NEUROTOXICITY STUDIES ON TWO AIR FORCE DEVELOPMENTAL OILS

The Toxic Hazards Research Unit was requested to evaluate the neurotoxic potential of two developmental oils, ML076-48 and ML078-80. The two compounds are perfluorinated oils which are candidate high temperature fluids for use in aircraft.

Many organophosphorus compounds have been found to cause delayed neurotoxic effects in man (Doull et al., 1979). A single exposure to a neurotoxic organophosphorus compound has been reported capable of producing axonal damage after a delay of eight to ten days. Low level nerve injury may occur in humans after chronic exposure to these compounds. Similar neurotoxic effects have been demonstrated in adult chickens and cats after exposure to triortho-cresylphosphate (TOCP) by Bareford and Glees (1963).

Although neither fluid is an organophosphate, the study was designed to determine if delayed neurotoxic effects result from exposure of adult chickens to a single peroral dose of the test oils. A vehicle control as well as a TOCP positive control were tested concurrently with the test compounds. Final determination of injury effect was based on a comparison of the test chickens with the TOCP control chickens.

Materials and Methods

Animals

Leghorn hens (Gallus domesticus, Carey Nick; 300-320 hybrid) five to seven months of age and weighing between 1.25 and 3.0 kilograms were purchased from Carey Farms, LaRue, Ohio. The debeaked hens were group housed in three by six foot pens to allow for freedom of movement. Food and water were available ad libitum.

Test Materials

Samples of the perfluorinated oils were supplied by the Air Force. Structures of the two developmental fluids are shown as follows:

MLO 76-48 - $F\left(-\underset{\underset{CF_3}{|}}{CFCF_2O}-\right)_n R_f$

MLO 78-80 - $R_fO\left(-CF_2CF_2O-\right)_m\left(-CF_2O-\right)_n R_f$

R_f= CF_3 or C_2F_5

LD_{50} Determination

Preliminary to the neurotoxicity testing, the acute oral toxicity of the two oils was determined. Oral intubation was accomplished employing a syringe fitted with a six inch infant catheter. The hens were weighed individually to determine the proper dose volume. None of the hens dosed with 5 mg/kg of either fluid died during the 14 day postexposure period of observation.

Acute Delayed Neurotoxicity Testing

The perfluorinated oils, as well as the positive control TOCP, were administered tc unfasted hens in an undiluted state. A negative control group received appropriate doses of corn oil. The method follows the proposed guidelines of the Environmental Protection Agency. The following dosing regimen was followed:

MLO76-48: A group of ten hens received a peroral dose of 5 ml/kg.

MLO78-80: A group of ten hens received a peroral dose of 5 ml/kg.

TOCP: A group of ten hens received a peroral dose of 500 mg/kg.

Corn Oil: A group of ten hens received a peroral dose of 1 ml/kg.

Grading by three observers began on the day following dosing and continued through twenty-one days after the initial dose.

The following point score was used:

Symptom Free.....0 Points
Doubtful of Minor Symptoms.....2 Points
Positive Paralytic Symptoms.....8 Points
Advanced Paralytic Symptoms.....12 Points
Death.....16 Points

During observation and grading, the hens were removed from their enclosure and placed on a rubber mat to provide sure footing. Symptoms of test hens noted during the observation period were compared with those seen in the TOCP treated hens. All hens were weighed prior to dosing and once weekly thereafter.

All test and control hens were examined for gross pathology at death. Longitudinal and cross sections of the spinal cord (cervical, lumbar, and thoracic regions) and a section of the sciatic nerve were sampled for histopathology examination.

Results

Mean body weights of the hens during the course of the study are shown in Table 71. All hens, including the corn oil controls, show a mean weight loss after one week. The initial weight loss is a result of randomizing the hens into different pens as well as beginning the daily routine of removing the hens to a new environment for the observations.

TABLE 71. EFFECT OF ORAL INTUBATION OF PERFLUORINATED OILS ON CHICKEN BODY WEIGHT (kg)

	Treatment Group			
Time Period	Corn Oil (Mean+SE)	TOCP (Mean+SE)	MLO 76-48 (Mean+SE)	MLO 78-80 (Mean+SE)
Pre dosing	1.58 ± 0.05	1.50 ± 0.03	1.55 ± 0.06	1.69 ± 0.07
1 Week	1.23 ± 0.66)	1.08 ± 0.05	1.15 ± 0.06	1.23 ± 0.07
2 Weeks	1.57 ± 0.04	1.36 ± 0.05	1.50 ± 0.06	1.65 ± 0.05
3 Weeks	1.70 ± 0.05	1.22* ± 0.07	1.63 ± 0.05	1.74 ± 0.05

*Different from controls at the 0.01 level of significance

After the routine had been established, normal gains were seen in all but the TOCP group. The observations of the corn oil control group as well as both perfluorinated oil groups resulted in all negative or doubtful scores. Positive neurotoxic symptoms were observed in all hens receiving TOCP.

Microscopic examination of the spinal cord and sciatic nerve tissue has not yet been completed.

Discussion

Under the conditions of this test the perfluorinated oils identified as MLO 76-48 and MLO 78-80 do not present a hazard as neurotoxic agents. The high dose level tested is the equivalent of a 70 kilogram man drinking more than 10 ounces of the fluid, an event which would be highly unlikely bv accident. If man's response to the perfluorinated oils parallels that of the hens, no neurotoxic hazard would be expected for military or civilian personnel involved in the manufacture or handling of the compounds.

EVALUATION OF NEUROTOXIC EFFECTS OF O-ETHYL-O'-(2-DIISOPROPYLAMINOETHYL)METHYLPHOSPHONITE

The Toxic Hazards Research Unit of the Department of Community and Environmental Medicine, University of California, Irvine, was requested to evaluate the neurotoxic potential of the Army chemical O-ethyl-O'-(2-diisopropylaminoethyl)methylphosphonite (EDMP). The chemical is an intermediate in the formation of O-ethyl-S-(2-diisopropylaminoethyl)methylphosphonothionate.

Because of the possibility of exposure of military and civilian personnel to EDMP during manufacturing, processing, or transportation, the Army has an interest in characterizing its acute and subchronic toxicity for setting occupational health standards. Previous studies have not had adequate characterization of the chemical nature of this material nor had the possibility of delayed neurotoxic effect been tested.

Many organophosphorus compounds cause delayed neurotoxic effects in man (Doull et al., 1979). A single exposure to a neurotoxic organophosphorus compound has been reported capable of producing axonal damage after a delay of eight to ten days. Low level nerve injury may occur in humans after chronic exposure to

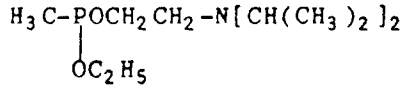
these compounds. Similar neurotoxic effects have been demonstrated in adult chickens and cats after exposure to triorthocresylphosphate (TOCP) by Bereford and Glees (1963).

This study was designed to determine if delayed neurotoxic effects result from exposure of adult chickens to peroral doses of EDMP. A vehicle control as well as a TOCP positive control was tested concurrently with EDMP. Final determination of an injury effect will be based on a comparison of the test chickens with the TOCP control chickens.

Materials and Methods

The chickens used in this study were as described under the previous study of Air Force developmental oils and the same methodology was also followed except that a second method was used in addition to the standard test.

Test Agent: O-(2-diisopropylaminoethyl)-O-ethyl methylphosphonite (EDMP)



The compound was supplied by the Army. The material was analyzed by gas chromatography/mass spectrometry to characterize its constitution. A chromatogram of a EDMP sample is shown in Figure 25.

Methods

Preliminary to neurotoxicity testing, the acute oral LD_{50} of EDMP in hens was determined. The compound was administered orally to nonfasted hens, three per concentration level and the LD_{50} calculated by the probit method of Finney (1971). Deaths which occurred during the 14 days immediately following the administration of the single dose were included in the final mortality tally.

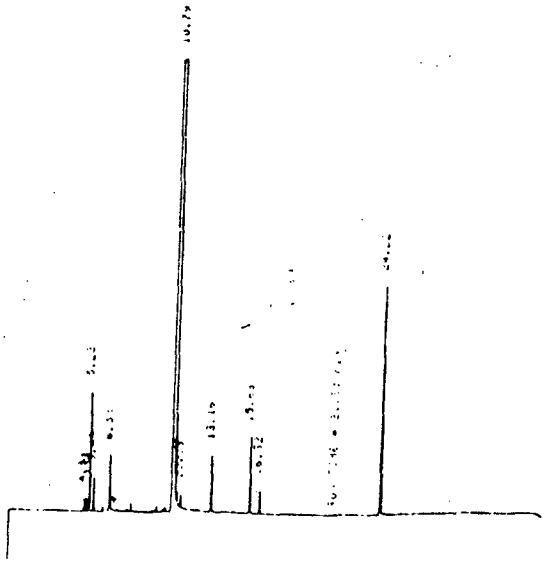


Figure 25. Gas chromatogram of O-ethyl-O'-(2-diisopropylaminoethyl) methylphosphonite .

Two methods of acute delayed neurotoxicity testing were used in this study. The first method, used by the U. S. Navy for military specification testing of fire resistant hydraulic fluids containing triaryl phosphate compounds, requires oral administration of the compound daily for five consecutive days.

This method has been described in previous sections of this report.

At the conclusion of the first study method used, nerve tissue was removed from the following hens: four corn oil controls, TOCP (3-90 mg/kg/day, 1-60 mg/kg/day), and EDMP (4-136 mg/kg/day). Samples of nerve tissue from the cervical, thoracic, and lumbar regions of the spinal cord as well as a sample of peripheral nerve (sciatic) were taken for microscopic examination.

The second method used was that which is detailed in the Environmental Protection Agency Proposed Guidelines for Pesticide Registration (CFR 1978). The hens were given a single oral dose of the compound and observed daily for twenty-one days.

The test substance was administered in the same manner as described previously with TOCP being used as a positive control. The dosing regimen was as follows:

EDMP - Fifteen hens were given a single peroral dose of 589 mg/kg.

TOCP - Ten hens were given a single peroral dose of 500 mg/kg.

Corn Oil - Ten hens were given a single peroral dose of 1 ml/kg.

Observations and grading by three technicians began the day following the peroral dose and continued through day twenty-one. Grading and scoring were done in the same manner as previously described. A TOCP equivalent calculation was made using the scores recorded on day 21. All hens were weighed prior to dosing and at weekly intervals during the observation period.

Moribund hens were killed to lessen the likelihood of unobserved death and subsequent autolysis. All hens were subjected to gross necropsy at death. A histopathologic examination of multiple longitudinal and cross sections of the spinal cord (cervical, lumbar, and thoracic regions) and sciatic nerve was made following necropsy.

Results

Acute LD_{50} Toxicity

Groups of three hens were given single peroral doses of EDMP with the results shown in Table 72. Deaths were delayed up to twelve days following intubation of the single dose. Symptoms included diarrhea, poor coordination, leg weakness, ataxia, and prostration. The 589 mg/kg level (slightly below the LD_{50}) resulted in one hen dying at nine days, another showed no symptoms during the observation period, and a third showed neurotoxic signs but had recovered by twenty-one days.

Two groups of three hens each were given 453 mg/kg or 227 mg/kg, respectively, of EDMP for five consecutive days as a range finder for the Navy neurotoxicity test. The three hens that received the high dose died between five and six days following the initial dose. The low level dosed hens all died between seven and eleven days after the initial dose. Symptoms observed in the hens were similar to those seen in the hens receiving a single peroral dose of the compound.

TABLE 72. RESULTS OF ACUTE ORAL DOSING OF ADULT HENS WITH EDMP

Dose Level (mg/kg)	14-Day Mortality Ratio (N = 3)	Days to Death
2156	3/3	4, 4, 5
1712	3/3	6, 7, 8
1359	3/3	7, 8, 8
906	2/3	9, 12
725	2/3	10, 12
589	1/3	9
453	0/3	-

LD_{50} and (95% confidence limits) = 707 (426-1151) mg/kg

Navy Method

Groups of unfasted hens were given single peroral doses of EDMP, TOCP, or corn oil for five successive days. The resultant scores twenty-one days following the initial dose are shown in Table 73. The vehicle control (corn oil) resulted in all negative scores while positive neurotoxic symptoms were observed in all hens given TOCP.

The two high dose levels of EDMP proved to be lethal to all hens. Two of each group were dead by the first scoring day with the remaining hens dying within the following two days. The next highest level had one hen that showed slight neurotoxic signs while the remaining three hens were free of neurotoxic signs. All hens from the remaining EDMP groups were symptom free during the observation period.

The hens dosed at 229 and 181 mg/kg EDMP proceeded rapidly through the classic symptoms of delayed neurotoxicity with most dying prior to reaching an advanced paralytic stage. The high mortality in the two high level groups and the lack of symptoms in 75% of the 136 mg/kg group preclude the validity of a TOCP equivalency calculation.

TABLE 73. SCORING OF NEUROTOXIC EFFECTS OBSERVED 21 DAYS FOLLOWING INITIAL PERORAL DOSE OF EDMP, TOCP, AND CORN OIL

Compound	Dose (mg/kg)	Group Score
EDMP	225	64.0[a]
	180	64.0[b]
	135	3.3
	90	0.0
	45	0.0
TOCP	90	36.0
	75	40.0
	60	34.0
Corn Oil	1[c]	0.0

[a]All hens in group died; deaths occurred between days seven and eight after initial dose.
[b]All hens in group died; death occurred between days 7 and 10 after initial dose.
[c]ml/kg

EPA Method

Mean body weights of the hens during the course of the study are shown in Table 74. All hens, including the corn oil controls, show a mean weight loss after one week. The weight loss may have resulted from randomizing the hens into different pens as well as beginning the daily routine of removing the hen to a new environment for the observations. After the routine had been established, normal gains were seen in the corn oil control group. Subnormal gains were observed in the TOCP positive control and the EDMP groups.

The mean scores of three observers for each chicken group are shown in Tables 75 to 77. The mean scores for each hen in the group are added to give a total daily score for each compound. The corn oil control group resulted in all negative or doubtful scores while positive neurotoxic symptoms were observed in all hens receiving TOCP.

TABLE 74. EFFECT OF ORAL INTUBATION OF EDMP ON CHICKEN BODY WEIGHT (Kg)

Time Period	Corn Oil (Mean + SE)	TOCP (Mean + SE)	EDMP (Mean + SE)
Pre Dosing	1.58 ± 0.05	1.50 ± 0.06	1.53 ± 0.03
1 Week	1.23 ± 0.07	1.08 ± 0.05	0.84* ± 0.08
2 Weeks	1.57 ± 0.04	1.36 ± 0.05	1.27* ± 0.07
3 Weeks	1.70 ± 0.05	1.22* ± 0.07	1.41* ± 0.04

* Different from corn oil controls at the 0.01 level of significance.

TABLE 75. NEUROTOXIC SYMPTOM SCORING RECORD OF HENS RECEIVING SINGLE DOSE OF 1 ML/KG CORN OIL

Days after Initial Dose	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Date Feb.	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Chicken No.	OBSERVATIONS - POINT SCORE																				
20.610	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
615	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
625	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
628	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
635	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
638	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
643	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
644	0	0	0.7	0.7	0	0	0	0	0	0	0	0	0.7	0.7	1.3	1.3	0	0	0	0	0
648	0	0	0	0	0	0	0	0	0	0	0.7	0.7	0.7	0	0.7	0	0	0	0	0	0
669	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Score	0	0	0.7	0.7	0.7	0	0	0	0	0	0.7	0.7	1.4	0.7	2.0	1.3	0	0	0	0	0

An EDMP hen died abruptly on the sixth day of the study without showing any positive neurotoxic signs. Because this death may have been due to extraneous causes, this animal's values were not included in the total scores. The remaining hens that died all showed positive neurotoxic signs and were included in the final scoring. To provide a score for the fourteen EDMP hens which could be compared to the ten TOCP hens, the total EDMP score was multiplied by 10/14.

TABLE 76. NEUROTOXIC SYMPTOM SCORING RECORD OF HENS RECEIVING A SINGLE DOSE OF 500 MG/KG TOCP

Days after Initial Dose	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Date: Feb.	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Chicken No.	OBSERVATIONS - POINT SCORE																				
20,609	0	0	0	0.7	0	0	0	0	0	0	3.3	8	8	8	8	8	8	10.7	12	12	12
612	0	0	0	0	0	0	0	0	0	3.3	8	8	8	12	12	12	12	12	12	12	16
624	0	0	0	1.3	0	0	0	0	0	2	4	4	8	8	8	8	8	8	8	8	12
627	0	0	0	0.7	0.7	0.7	0	0	0.7	1.3	6	8	8	8	8	8	12	12	12	12	12
646	0	0	0.7	2	6	16	Dead	→													
654	0	0	0	0	0	0	0	0	0	0	0	8	8	8	8	8	8	8	8	8	8
656	0	0	0	0	0	0	0.7	0	0	0.7	4	8	8	8	8	8	8	10.7	10.7	12	12
657	0	0	0.7	1.3	0.7	0	0	0	0	0	4	6	8	8	8	8	8	10.7	12	12	12
660	0	0	0	0	0	0	0	0	0	0.7	0	1.3	4	2	6	4	6	2	6	8	6
665	0	0	0	0	0	0	0	0	0	0.7	0.7	4	6	8	8	8	8	8	12	12	10.7
Total Score	0	0	1.4	6	7.4	16.7	16.7	16	16.7	24.7	52	71.3	82	86	90	88	94	98.1	108.4	112	11.7

Six of the 14 hens showed negative or doubtful neurotoxic signs. However, of the remaining 8 hens, 5 showed advanced paralytic symptoms. The peak neurotoxic effect took place at 13 days when a total score of 79.5 was recorded. Comparing scores of EDMP and TOCP on the thirteenth day resulted in a TOCP equivalent of 82.3%. The results of this test indicate that EDMP is a delayed neurotoxin.

The discrepancy between the results of this test and that of the Navy method of testing EDMP appears to be the dose levels selected. The previous testing resulted in the two high levels causing total mortality.

Discussion

Under the conditions of the EPA testing methods, EDMP represents a TOCP equivalency of approximately 80% within two weeks of dosing. EDMP presents a neurotoxic hazard to adult chickens and, as such, appropriate measures should be taken to avoid oral contact of the compound by military and civilian personnel.

TABLE 77. NEUROTOXIC SYMPTOM SCORING RECORD OF HENS RECEIVING A SINGLE DOSE OF 589 MG/KG EDMP

Days after Initial Dose	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Date: Feb.	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Chicken No.	OBSERVATIONS - POINT SCORE																				
20,608	0	0	0	0	0.7	16	Dead →														
611	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
619	0	0	0	0	0	0.7	9.3	8	8	8	8	8	8	8	8	8	8	8	8	12	12
621	0	0	0	0.7	6	12	12	12	12	18	Dead →										
631	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0.7	0.7	0.7	0.8	0	0	0
633	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
636	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
645	1.3	1.3	1.3	6	6	8	12	8	8	8	9.3	12	9.3	9.3	10.7	8	8	8	8	8	8
647	0	0	0	8	16	Dead →															
650	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
655	0	0	0	0.7	0.7	4	8	16	Dead →												
659	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
661	0	0	0	0	0	0.7	1.3	8	8	8	10.7	12	12	10.7	12	9.3	12	12	12	12	10.7
663	0	0	0	0	0	0.7	1.3	8	8	8	9.3	12	12	9.3	10.7	9.3	8	8	8	8	8
666	0	0	0	0	0	0.7	6	8	6	8	12	12	12	12	16	Dead →					
Total Score	1.3	1.3	2.0	15.4	29.7	42.8	65.7	84	82.7	88	97.4	104	111.3	93.7	106.1	99.3	100.7	100.7	100	104	102.7
X 10/14 =	0.93	0.93	1.43	11	[illegible]	30.6	47.1	1.0	59.1	12.9	69.5	74.3	79.5	69.5	75.8	70.9	71.9	71.9	71.4	74.3	73.4

THE ACUTE ORAL, DERMAL, AND INTRAPERITONEAL TOXICITY AND THE SENSITIZATION AND IRRITATION PROPERTIES OF O-ETHYL-O'-(2-DIISOPROPYL-AMINOETHYL)METHYLPHOSPHONITE

Because of the possibility of exposure of military and civilian personnel to EDMP during manufacturing, processing, or transportation, the Army has a strong interest in characterizing its acute and subchronic toxicity. Previous experiments have been conducted to determine the effects of acute inhalation exposure to EDMP (Dimmick, Jr. et al., 1979). Exposure to 12,300 mg/m^3 for one hour and 2,350 mg/m^3 for 6 hours failed to produce 50% mortality in rats and guinea pigs. Intravenous (IV) administration yielded an LD_{50} of 203.7 mg/kg in mice and 164.4 - 207.5 mg/kg in rabbits. The IV LD_{50} values would classify this material as toxic but not highly toxic. McNamara et al. (1981) performed subchronic inhalation studies of 27 weeks at 5.0 and 22.4 mg/m^3. Experimental animals exposed were Sprague-Dawley/Wistar rats, ICR Swiss and "A" strain mice, and Hartley guinea pigs. Measurements were made of hematologic and clinical chemistry parameters, pulmonary resistance in guinea pigs, spontaneous activity in rats, and organ pathology.

The unstable character of EDMP was demonstrated in the subchronic inhalation studies in which analyzed concentrations ranged from 13-41% of nominal. At least one of the identified breakdown products, O-diisopropylaminoethylmethylphosphinate, was considered to be highly toxic and may give misleading toxicity results if present in excessive quantities in the EDMP used for experimentation.

Because previous studies have not had adequate characterization of the chemical nature of the material delivered to the test animals, uncertainty still exists about the acute effects of exposure to EDMP. This study was designed to conduct a series of acute tests by various routes. Prior to and during these studies, the test material was analyzed and characterized to provide qualitative and quantitative information about the material to which animals were exposed. Information concerning these chemical characterization studies is given in Section III of this report.

EDMP was supplied by the U. S. Army in two 20 gallon drums. Upon receipt, it was analyzed by gas chromatography (G.C.) and by gas chromatography/mass spectrophotometry. It was distributed into 2 oz brown bottles under nitrogen for use. Each time a bottle was opened for use it was analyzed by G.C. to insure that no substantial changes had occurred.

Methods

Oral Toxicity

EDMP was suspended in corn oil. Glass syringes with oral dosing needles were used to administer the compound to rats and mice. Animals were fasted for at least 16 hours prior to administration of the oral dose. The dose volume for the test animals was 0.01 ml/gm body weight, and the animals were weighed individually at the time of dosing. Male and female rats and male and female mice were used. Ten animals of each sex were dosed at each level, and the LD_{50} values with 95% confidence limits were calculated using the probit method of Finney (1971). Deaths which occurred during the 14 days immediately following the administration of the single dose were included in the final mortality tally. Surviving animals were weighed at 1, 2, 4, 7, 10, and 14 days postexposure and killed on the 14th day postexposure. Symptoms were recorded on appropriate forms.

In addition, 10 rats and 50 mice of the most sensitive sex were orally exposed to an LD_{10}. Blood was taken from the orbital plexus of the rats prior to dosing and at 1 hr, 24 hrs, 48 hrs, 7 days, and 15 days after dosing for cholinesterase determinations. Five animals were bled at the 1/2 hour and 24 hr sampling times. The remaining animals were bled at 48 hrs and all animals were bled at 7 and 15 days after treatment. The number of mice in test and control groups was sufficient to allow blood collection at 4 hrs, 24 hrs, 48 hrs, 7 days, and 15 days post-treatment.

Intraperitoneal Toxicity

Samples were prepared as for oral toxicity. Glass syringes with 20 gauge needles were used to administer injection volumes of 0.01 ml/gm body weight.

The animals were weighed individually just prior to dosing to determine the proper injection volume for male and female rats and mice. Ten rats and ten mice of each sex were dosed at each level. The LD_{50} values with 95% confidence limits were calculated using the probit method of Finney (1971). Surviving animals were weighed at 1, 2, 4, 7, 10, and 14 days postexposure and killed on the 14th postexposure day. Symptoms were recorded. Cholinesterase determinations were conducted in the same manner as for the oral test.

Dermal Toxicity

Male and female albino New Zealand rabbits weighing between 2 and 3 kilograms were used for dermal toxicity studies (five animals of each sex were tested at each dose level). All rabbits were clipped as closely as possible to prevent the fur from contaminating the laboratory. The backs and sides down to about halfway to the abdomen were clipped from the saddle area to the hind legs of the rabbits.

The animals were individually weighed prior to dosing to determine the proper volume. The appropriate volume of liquid material was applied undiluted to the back of the rabbit and was divided as equally as possible between the two sides. The dose was kept in place by applying 8 ply gauze patches over the liquid on each side of the back. A patch of plastic wrap was applied over the entire clipped back area and elastoplast tape was used to wrap the entire midsection of the rabbit to keep the dose in place. Specially designed rabbit restraining harnesses were fitted to each rabbit at the time of dosing and kept in place during the entire contact

period. These harnesses prevented excessive movement and prevented the rabbit from chewing on the taped area. The harnesses allowed for access to food and water during the dosing period.

All doses were kept in contact with the rabbit's skin for 24 hours. After 24 hours, the tape, plastic wrap, and gauze were removed and the harnesses taken off. The rabbits were maintained in individual cages postexposure and observed for mortality or other signs of toxicity for 14 days postexposure. Any deaths that occurred during this period were included in the final tally.

Eye Irritation

One-tenth milliliter of undiluted EDMP liquid was applied to one eye of each of 9 albino rabbits. The opposite eye was untreated and served as a control. The eyes of the test animals were examined with fluorescein stain prior to use to ensure absence of lesions or injury. The treated eyes of 6 rabbits remained unwashed while the remaining 3 rabbits received test material and had the treated eyes flushed for one minute with lukewarm water starting no sooner than 20-30 seconds after instillation. Examination for gross signs of eye irritation were made at 1, 2, 3, 4, and 7 days following application. Scoring of irritative effects was according to the method of Draize (1959) in which corneal, iris, and conjunctival effects are scored separately.

Skin Irritation

A patch-test method was used to determine the degree of primary skin irritation of intact and abraded skin of albino rabbits.

Six rabbits were clipped of all possible hair on the back and flanks 24 hours prior to exposure to allow for recovery of the skin from any abrasion resulting from the clipping. One of two areas on the back was abraded by making minor incisions with a syringe needle through the stratum corneum, but not deep enough to disturb the dermis or to produce bleeding. These were made in a square pattern.

A volume of 0.5 ml EDMP fluid was applied to the designated patch areas and covered by a 1-inch square of surgical gauze 2 layers thick. The gauze patches were held in place with strips of elastoplast tape. The entire area was covered with a strip of plastic wrap and secured with more elastoplast tape. These patches remained in place on the rabbits for 24 hours. During that time, the rabbits were fitted with leather restraining collars. These

collars prevented disturbance of the patch area, while allowing the rabbits freedom of movement and access to food and water during the test period. After 24 hours, the wrap and patches were removed and the test areas evaluated for irritation using the Draize (1959) table as a reference standard. A combined score was recorded. Readings were also made at 72 hours (48 hours after the first reading). If injury occurred, scoring was continued three times a week until injury subsided or was deemed irreversible.

Skin Sensitization

A study performed in this laboratory by Horton et al. (1981) compared three widely used skin sensitization techniques using a strong sensitizer, a weak to moderate sensitizer, and a non-sensitizer as test agents. These studies confirmed what had been indicated in private communications that the modified Maguire Test as used by the Dow Chemical Company was the most sensitive of the three. Therefore, this test has replaced the Landsteiner Test in this laboratory for the determination of skin sensitization potential.

Ten female albino guinea pigs, Hartley strain, 6 to 8 weeks of age, were used. EDMP was tested for primary irritation on 3 guinea pigs by application to the clipped flank. Observation was at 24 hours for signs of irritation. The maximum non-irritating concentration of EDMP in petrolatum was found to be 5%. The sensitization test was conducted at this concentration. An area on the back of each animal directly above the forelegs was clipped with electric clippers and chemically depilated with a commercial product on the morning of the first insult exposure. Test solutions, 0.1 ml at each application, were applied to this area on a 1/2 x 1/2 inch cotton gauze square, covered with plastic wrap, and held in place with adhesive tape. The first insult patch was allowed to remain in place for two days, then removed, and a second application of 0.1 ml was made. Two days later, this patch was removed, a total of 0.2 ml of a 50% aqueous dilution of Freund's* adjuvant per animal was injected intradermally, using 2 or 3 points adjacent to the insult site, then a new patch of 0.1 ml of the test material was applied. On the third day after this application, the patch was removed and a new patch of 0.1 ml of the material was applied. The last patch was removed 2 days later, and the animals were allowed to rest for 2

* Bacto Adjuvant Complete, Freund, Difco Laboratories, Detroit, Michigan.

weeks. Each time the insult patches were removed, the condition of the skin at the application site was evaluated and recorded. When the last patch was removed, the toes of the hind feet of each animal were taped to prevent the animal from scratching the irritated area. About 2 inches of 1/2 inch tape was wrapped around each foot so that no toenails protruded.

After the two week rest period, both flanks of the animals were clipped and challenged on one side with the test solution and petrolatum on the other flank. The challenge applications were not occluded. The skin response at these sites was recorded at 24 and 48 hours after application. Any animal showing measurable erythema and/or edema at the challenge site was rated as a positive responder according to the following grading system.

Erythema	Edema
0 - None	0 - None
1 - Very slightly pink	1 - Very slight
2 - Slight pink	2 - Slight
3 - Moderate red	3 - Moderate
4 - Very red	4 - Marked

In scoring the Maguire Test, the important statistic is severity of the reaction. The following table is used to classify test materials as to sensitization potential.

Sensitization Rate (%)	Sensitization Potential Grade	
10	I	Weak
20-30	II	Mild
40-60	III	Moderate
70-80	IV	Strong
90-100	V	Extreme

Test Animals

Male and female Sprague-Dawley derived rats, (weighing 200-300 grams and 150-250 grams, respectively), were obtained from Charles River Breeding Labs, Wilmington, Mass. Male and female New Zealand white rabbits weighing 2 to 3 kg were obtained from Davis Rabbitry or bred in-house. Female Hartley strain guinea pigs weighing 300 to

500 grams were obtained from Murphy Breeding Labs, Plainfield, Indiana. Male and female mice 9 to 11 weeks of age were obtained from Charles River Breeding Labs, Wilmington, Mass.

Results

Acute Oral: The acute oral LD_{50} values for male rats and male and female mice were between 2.35 and 2.75 g/kg. Female rats were more sensitive to orally administered EDMP with an LD_{50} value of 0.71 g/kg (Table 78).

Acute Intraperitoneal: The acute intraperitoneal LD_{50} values of EDMP for male and female rats and mice were between 0.27 and 0.52 g/kg. Rats were slightly more resistant than mice, and female mice were the most susceptible as shown in Table 79.

Acute Dermal: The acute dermal LD_{50} values of EDMP for male and female rabbits were 1.68 and 1.90 g/kg, respectively (Table 80).

Skin and Eye Irritation: EDMP did not cause any eye irritation. However, 3 of 6 rabbits demonstrated irreversible skin damage as shown in Table 81, and EDMP may be catagorized as "corrosive" (Table 81).

Skin Sensitization: Skin sensitization tests with EDMP treated guinea pigs were negative. EDMP is not a sensitizing material.

Cholinesterase: EDMP caused a reversible inhibition of female rat and mouse erythrocyte cholinesterase (Table 82). Rat erythrocyte cholinesterase was more depressed than that of mice. Plasma cholinesterase was significantly depressed in rats but not mice (as shown in Table 83). Significant depression is generally accepted to be any depression of 20% or greater. Rat plasma cholinesterase activity levels had returned to near pretreatment level 7 days after treatment. In summary, rat plasma and erythrocyte cholinesterase levels were more affected by oral and intraperitoneal administration of EDMP than were cholinesterase levels in mice.

TABLE 78. THE ACUTE ORAL TOXICITY OF SINGLE DOSES OF EDMP FOR SPRAGUE-DAWLEY RATS AND SWISS ALBINO MICE

Dose Level (g/kg)	14 Day Mortality No. Dead/No. Dosed	LD_{50} g/kg (95% C.L.)
	Male - Rat	
4.09	8/10	
3.18	6/10	
2.36	5/10	2.75 (2.36-3.35)
2.27	5/10	
2.09	3/10	
1.91	0/10	
	Female - Rat	
1.81	10/10	
0.91	6/10	
0.72	7/10	0.71 (0.52-0.89)
0.54	2/10	
	Male - Mouse	
2.72	7/10	
2.45	6/10	2.36 (1.90-2.81)
2.36	5/10	
1.81	2/10	
0.91	0/10	
	Female - Mouse	
4.09	10/10	
3.18	6/10	
2.27	4/10	2.35 (1.87-2.87)
1.82	4/10	
0.82	0/10	

TABLE 79. THE ACUTE INTRAPERITONEAL TOXICITY OF SINGLE DOSES OF EDMP FOR SPRAGUE-DAWLEY RATS AND SWISS ALBINO MICE

Dose Level (g/kg)	14 Day Mortality No. Dead/No. Dosed	LD_{50} g/kg (95% C.L.)
	Male - Rat	
0.72	9/10	
0.57	9/10	0.52 (0.46-0.57)
0.45	2/10	
0.36	0/10	
	Female - Rat	
0.91	9/10	
0.72	10/10	0.51 (0.43-0.57)
0.54	6/10	
0.45	5/10	
0.36	0/10	
	Male - Mice	
0.54	10/10	
0.45	7/10	
0.36	4/10	0.39 (0.34-0.43)
0.27	0/10	
0.18	0/10	
	Female - Mice	
0.54	10/10	
0.36	10/10	
0.27	4/10	0.27 (0.24-0.31)
0.23	2/10	
0.18	0/10	

TABLE 80. ACUTE DERMAL TOXICITY OF EDMP FOR NEW ZEALAND WHITE RABBITS

Dose Level (g/kg)	14 Day Mortality No. Dead/No. Dosed	LD_{50} g/kg (95% C.L.)
	Male - Rabbit	
1.91	5/5	
1.77	5/5	
1.72	8/10	
1.63	2/5	1.68 (1.62-1.73)
1.59	0/5	
1.54	1/5	
1.36	0/10	
	Female - Rabbit	
2.36	5/5	
2.08	4/5	1.90 (1.63-2.08)
1.95	3/5	
1.91	1/5	

TABLE 81. PRIMARY SKIN IRRITATION OF EDMP

Rabbit Number	G 19		G 94		H 00		E 43		F 12		H 14	
	I[1]	A[2]	I	A	I	A	I	A	I	A	I	A
Erythema[3]												
24 hrs	1	1	1	1	0	0	1	1	1	1	1	1
72 hrs	1	1	1	1	1	1	1	1	1	1	1	1
Day 5	2	2	1	1	2	2	1	1	2	2	1	1
Day 7	1	1	1	1	2	2	0	0	0	0	1	1
Day 9	0	0	0	0	0	0	0	0	0	0	0	0
Day 12	0	0	0	0	0	0	0	0	0	0	0	0
Edema[3]												
24 hrs	1	1	1	1	1	1	2	1	1	1	1	1
72 hrs	1	1	1	0	1	1	0	1	0	0	0	0
Day 5	1	2	0	1	0	0	0	0	0	0	0	0
Day 7	0	0	0	0	2	2	0	0	0	0	1	1
Day 9	0	0	0	0	0	0	0	0	0	0	0	0
Day 12	0	0	0	0	0	0	0	0	0	0	0	0
Necrosis[4]												
24 hrs	0	0	0	0	0	0	0	0	0	0	0	0
72 hrs	0	5	0	0	5	5	0	5	0	0	0	0
Day 5	0	5	0	0	5	5	0	5	0	0	0	0
Day 7	0	5	0	0	5	5	0	5	0	5	0	0
Day 9	*	5	*	*	5	5	*	5	*	*	*	*
Day 12	*	5	*	*	5	5	*	5	*	*	*	*

*Eschar Formation

[1]I = Intact [2]A = Abraded

[3]Erythema & Edema are scored on a scale of 1-4 with 4 being the most severe.

[4]Necrosis is scored 0, 5, 10, or 15 with 15 being the largest area of necrosis

TABLE 82. EFFECT OF EDMP ON ERYTHROCYTE CHOLINESTERASE IN RODENTS

Species	Sex	Route of Administration	Dose (g/kg)	% of Pretreatment Activity					
				Pre Treatment	Post Treatment				
					0.5 Hrs	4 Hrs	24 Hrs	48 Hrs	7 Days	15 Days
Rat	F	Oral	0.45	100%		46	33	18	80	66
Rat	F	I.P.	0.36	100%	34		17	22	87	92
Mouse	F	Oral	1.4	100%	73		50	42	94	83
Mouse	F	I.P.	0.23	100%	69	53	64	78	112	105

TABLE 83. EFFECT OF EDMP ON PLASMA CHOLINESTERASE IN RODENTS

Species	Sex	Route of Administration	Dose (g/kg)	% of Pretreatment Activity						
				Pre Treatment	Post Treatment					
					0.5 Hrs	4 Hrs	24 Hrs	48 Hrs	7 Days	15 Days
Rat	F	Oral	0.45	100%		70	49	43	83	92
Rat	F	I.P.	0.36	100%	50		25	44	86	111
Mouse	F	Oral	1.4	100%	101		88	87	113	115
Mouse	F	I.P.	0.23	100%	90	104	94	97	95	99

EVALUATION OF THE ACUTE TOXICITY, IRRITATION, AND SENSITIZATION POTENTIAL OF TWO ANTIMONY THIOANTIMONATE MATERIALS

Introduction

The U. S. Navy is currently using a graphite-based material on aircraft carriers as a lubricant for airplane catch cables. These cables are stretched across the flight deck and are snagged by the arresting hook on the tail of incoming aircraft. The performance characteristics of the graphite lubricant are adequate; however, clean-up is difficult and the slippery deck creates a safety hazard. Therefore, the Navy is evaluating a new lubricant which has a basic composition of 3-5% antimony thioantimonate (ATA) in calcium cup grease #3. This lubricant is undergoing performance testing at the Naval Air Engineering Center. A partial acute toxicologic evaluation of the technical material had been conducted by the manufacturer, Pennwalt Corporation.

The only effects reported after dermal administration of 2000 mg/kg technical ATA to rabbit skin were varying degrees of erythema and edema (Wolfe, 1981). In irritation studies, the material was found to cause slight edema dermally and slight reversible eye irritation in unwashed rabbit eyes [1,2]. There were no effects noted in male and female rats after oral administration of 5000 mg/kg technical ATA (Wolfe, 1981a). A search of the literature revealed numerous publications on the toxicity to humans of various antimony compounds used as parasiticides. The primary reported effects of

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[1] Latven, A. R. (1981), Antimony thioantimonate, skin irritancy in rabbits, Pharmacology Research, Inc., Unpublished report.

[2] Latven, A. R. (1981a), Antimony thioantimonate, skin irritancy in rabbits, Pharmacology Research, Inc., Unpublished report.

these materials were cardiac in nature with electrocardiographic (ECG) changes in the T-wave configuration. The T-wave changes varied from a minor reduction in amplitude to deep T-wave inversions. In one study, these changes occurred in 100% of patients dosed with tartar emetic, a trivalent antimonial compound (Schroeder et al., 1946). In another study, SGOT determinations as well as ECG evaluations were done on patients undergoing antimonial compound therapy. There were dose dependent elevations of SGOT and several instances of T-wave effects, but no correlation could be established between the two observations (Waye et al., 1962).

NMRI/TD requested that UCI/THRU further evaluate the toxicity of technical ATA and the cup grease formulation.

Test Materials

Antimony Thioantimonate, Technical

Manufacturer:	Pennwalt Corporation
Batch No.:	5363-93-1
Physical Appearance:	Burnt orange powder
Chemical Formula:	$SbSbS_4$
NMRI/TD No.:	2054-1

Full Formulation (3-5% Antimony Thioantimonate in Calcium Cup Grease

Manufacturer:	Pennwalt Corporation
Batch No.:	565D-5-1
Physical Appearance:	Reddish-orange grease
Chemical Family:	Calcium Soap - thickened petroleum hydrocarbons with 5% $SbSbS_4$
NMRI/TD No.:	2281-1

Calcium Cup Grease No. 3

Manufacturer:	Cook's Industrial Lubricants, Inc.
Boiling Point, °F:	545
Vapor Pressure, (mmHg):	<0.01
Specific Gravity:	0.9
Physical Appearance:	Light amber grease
Chemical Family:	Calcium soap - thickened petroleum hydrocarbons
NMRI/TD No.:	2054-2

Materials and Methods

Intraperitoneal Toxicity

Agglomerates of technical antimony thioantimonate were eliminated prior to use by sieving. Appropriate amounts of the test material were suspended in distilled water and injected intraperitoneally with a syringe and large gauge needle. Dose groups consisted of 10 male and 10 female Sprague-Dawley rats weighing 200-300 grams and 150-200 grams, respectively. Dose volumes equivalent to 1% of the animal's body weight were administered.

At least 4 dose levels producing mortality sufficient to calculate a 14 day LD_{50} with 95% confidence limits of 20% or less were tested. The LD_{50} value was calculated using the method of Finney (1971).

Mortality was recorded for 14 days after dosing. Animals were observed frequently on the day of dosing and twice daily during the 14 day observation period. Signs of toxicity were also recorded. Body weights were obtained at the time of dosing and at 1, 2, 4, 7, 10, and 14 days post treatment. All animals were examined by gross necropsy following death.

Eye Irritation

One-tenth gram of the finely ground test material was applied to one eye of each of twelve albino rabbits. The opposite eye was untreated and served as a control. The treated eyes of 6 rabbits remained unwashed. The treated eyes of the remaining rabbits were irrigated for one minute with lukewarm water starting no sooner than 20-30 seconds after instillation. Examinations for gross signs of eye irritation were made at 24 and 72 hours post-application.

Scoring of irritative effects was according to the method of Draize (1959) in which corneal, iris, and conjunctival effects are scored separately.

Sensitization - Guinea Pigs

Ten female albino guinea pigs, Hartley strain, 6 to 8 weeks of age, were used for each material (technical ATA, full formulation ATA and Ca cup grease). The Maguire method was used (Maguire, 1973).

Technical ATA was suspended in liquid petrolatum and the grease formulations were applied undiluted. An area on the back of each animal directly above the forelegs was clipped with electric clippers and chemically depilated with a commercial depilatory on the morning of the first insult exposure. At each application, 0.1 gm of test material was applied to this area on a 1/2 x 1/2 inch cotton gauze square, covered with dental dam, and held in place with adhesive tape. The first insult patch was allowed to remain in place for two days, then removed, and a second application of 0.1 gm was made. Two days later, this patch was removed, a total of 0.2 ml of Freund's* 50% adjuvant per animal was injected intradermally, using 2 or 3 points adjacent to the insult site, then a new patch of 0.1 gm of the test material was applied. On the third day after this application, the patch was removed and a new patch of 0.1 gm of the test material applied. The last patch was removed two days later, and the animals were allowed to rest for two weeks. Each time the insult patches were removed, the condition of the skin at the application site was evaluated and recorded. When the last patch was removed, the toes of the hind feet of each animal were taped to prevent the animal from scratching the irritated area.

After the two-week rest period, both flanks of the animals were clipped and challenged on one side with the test material and the vehicle, if any, on the other flank. The challenge applications were not occluded. The skin response at these sites was recorded at 24 and 48 hours after application. Any animal showing measureable erythema and/or edema at the challenge site was rated as a positive responder according to the following grading system.

* Bacto Adjuvant Complete, Freund, Difco Laboratories, Detroit, Michigan.

Erythema	Edema
0 - None	0 - None
1 - Very slight pink	1 - Very slight
2 - Slight pink	2 - Slight
3 - Moderate red	3 - Moderate
4 - Very red	4 - Marked

In scoring the Maguire Test, the important statistic is frequency of the reaction. The following table is used to classify test materials as to sensitization potential.

Sensitization Rate (%)	Sensitization Potential Grade	
10	I	Weak
20-30	II	Mild
40-60	III	Moderate
70-80	IV	Strong
90-100	V	Extreme

Dermal Toxicity

A 90 day subchronic dermal toxicity study is scheduled to start in the fall of 1983. Results will be described in future annual reports.

Results

The results of the acute intraperitoneal toxicity, eye irritation, and skin sensitization tests are summarized in Table 84.

Intraperitoneal Toxicity: The acute intraperitoneal LD_{50} values of 445 mg/kg in male and 568 mg/kg in female rats indicate ATA Technical is moderately toxic as shown in Table 85.

Eye Irritation: ATA Technical was found to be a reversible eye irritant. The average irritation scores are presented in Table 86.

TABLE 84. SUMMARY OF THE ACUTE INTRAPERITONEAL TOXICITY, IRRITATION AND SENSITIZATION POTENTIAL OF ATA

Species	Sex	Formulation	Intraperitoneal LD_{50} (mg/kg) 95% C.L.	Eye Irritation	Skin Sensitization
Rat	M	Technical	445 (383-539)		
Rat	F	Technical	568 (446-675)		
Rabbit	*	Technical		Positive (Reversible)	
G. Pig	F	Technical			Negative
G. Pig	F	Full Formulation			Positive
G. Pig	F	Ca Cup Grease			Positive

*Both sexes used

TABLE 85. ACUTE INTRAPERITONEAL TOXICITY OF ATA TECHNICAL FOR SPRAGUE-DAWLEY RATS

Dose Level (mg/kg)	14 Day Mortality No. Dead/No. Dosed	LD_{50} (mg/kg) and 95% C.L.
	Male - Rat	
750	10/10	
500	5/10	445 (383-539)
350	4/10	
300	0/10	
	Female - Rat	
1000	9/10	
794	7/10	
630	6/10	568 (446-675)
500	7/10	
400	0/10	

All animals in both groups exhibited redness, chemosis, and discharge. However, the reactions were less in the washed eye group than in the unwashed eye group. The majority of the animals also exhibited a slight circumcorneal injection.

TABLE 86. PRIMARY EYE IRRITATION IN RABBITS TREATED WITH ATA TECHNICAL FORMULATION

Group	Mean Irritation Scores Day After Exposure 1	3	4	7	10	13
Eyes Not Washed After Instillation	23	15	9	4	2	0
Eyes Washed After Instillation	16	10	4	3	1	0

There was a very slight corneal opacity in 4 of the 6 rabbits of the "unwashed" group and 1 of 6 rabbits from the "washed" group at 7 days after instillation. This opacity was not discernible without the use of fluorescein dye and an ultraviolet light.

Skin Sensitization: ATA Technical demonstrated no sensitization potential. The full formulation, however, was found to have a high sensitization potential implying that the calcium cup grease may have been the sensitizing agent. Therefore, a second trial using the full formulation was conducted, and calcium cup grease was tested simultaneously. Results of the second trial confirmed the high sensitization potential of the full formulation. Furthermore, the cup grease was also found to have a high sensitization potential (Table 87). These animals were then cross-challenged in the following manner: animals sensitized to full formulation-challenged with ATA Technical and calcium cup grease, animals sensitized with calcium cup grease-challenged with ATA Technical and full formulation. None of the animals challenged with ATA Technical reacted. All animals challenged with the full formulation or calcium cup grease reacted (Table 88). These results indicate that some component of the calcium cup grease is the sensitizing agent.

Conclusions

The results of these experiments provide evidence that antimony thioantimonate is toxic to rats by intraperitoneal administration and is a reversible eye irritant in rabbits with effects disappearing by 13 days post treatment. Guinea pig sensitization tests prove that ATA alone is not a sensitizing agent but some undefined component of the calcium cup grease is an active sensitizer. Dermal contact is probably the greatest risk to personnel using the formulation. Allergic reaction to the full formation may result from repeated exposure in the absence of protective equipment.

TABLE 87. GUINEA PIG SENSITIZATION RESPONSE TO ATA TECHNICAL, ATA FULL FORMULATION AND CALCIUM CUP GREASE

24 Hrs Postchallenge

ATA Technical		ATA Full Formulation				Calcium Cup Grease	
		1st Trial		2nd Trial			
Test Site	Control Site	Test Site	Control Site	Test Site	Control Site	Test Site	Control Site
0	0	2	0	1	0	1	0
0	0	2	0	2	0	0	0
0	0	1	0	1	0	0	0
0	0	1	0	1	0	1	0
0	0	1	0	1	0	1	0
0	0	1	0	1	0	1	0
0	0	2	0	1	0	1	0
0	0	3	0	2	0	1	0
0	0	1	0	2	0	1	0
0	0	2	0	1	0	0	0
				(% Positive)			
0	0	100	0	100	0	70	0

48 Hrs Postchallenge

0	0	1	0	2	0	1	0
0	0	1	0	2	0	0	0
0	0	2	0	1	0	1	0
0	0	2	0	1	0	1	0
0	0	3	0	1	0	1	0
0	0	1	0	1	0	1	0
0	0	2	0	1	0	1	0
0	0	3	0	1	0	1	0
0	0	1	0	2	0	1	0
0	0	2	0	2	0	1	0
				(% Positive)			
0	0	100	0	100	0	90	0

TABLE 88. GUINEA PIG SENSITIZATION RESPONSE TO CROSS CHALLENGE

Animal Number	Sensitized To	Challenged With	24 Hr Evaluation	48 Hr Evaluation
1	Calcium Cup Grease	ATA Technical	0	0
2	" "	" "	0	0
3	" "	" "	0	0
		% Positive	0	0
15	Full Formulation	ATA Technical	0	0
16	" "	" "	0	0
17	" "	" "	0	0
		% Positive	0	0
4	Calcium Cup Grease	Full Formulation	1	1
5	" "	" "	1	1
6	" "	" "	1	2
7	" "	" "	1	2
8	" "	" "	1	2
9	" "	" "	2	2
10	" "	" "	1	2
		% Positive	100	100
18	Full Formulation	Calcium Cup Grease	1	2
19	" "	" "	2	2
20	" "	" "	2	2
23	" "	" "	2	2
24	" "	" "	2	2
25	" "	" "	1	2
26	" "	" "	2	2
		% Positive	100	100

SECTION III
FACILITIES

QUALITY CONTROL OF O-ETHYL-O'-(2-DIISOPROPYLAMINOETHYL) METHYLPHOSPHONITE

As a source of material for the scheduled series of experiments with EDMP, two 10-gallon stainless steel drums were received during July of 1982. Because of the unstable nature of EDMP, frequent quality control analyses were required to insure that no significant decomposition had taken place over the course of the experiments.

EDMP is subject to hydrolysis and disproportionation when exposed to moisture. Grula and Armelie (1976) reported the presence of the following impurities after addition of water to liquid samples of EDMP:

Ethanol
O,O'-Diethylmethylphosphonite
O-Ethylmethylphosphinate
Diisopropylaminoethanol
O-(Diisopropylaminoethyl)methylphosphinate
O-Ethyl-O'-(diisopropylaminoethyl)methylphosphonate
O-(Diisopropylaminoethyl)ethylmethylphosphinate
O-ethyl(diisopropylaminoethyl)methylphosphinate
O,O'-Bis(diisopropylaminoethyl)methylphosphonite

As the method of analysis, Grula and Armelie utilized gas chromatography using a 1/4" OD column packed with SE-52 on 80 to 100-mesh Gas Chrom Q. Since this laboratory is equipped with facilities for capillary chromatography, we decided to take advantage of the highly superior resolution of this method choosing a coating as similar as possible to the SE-52 used by Grula and Armelie.

Identical 50 meter SE-54 fused silica WCOT (Wall-Coated Open Tubular) columns from Hewlett-Packard were obtained. One was installed in a Varian 3700 gas chromatograph (GC) for quality control (QC) analysis, while the other was placed in the Hewlett-Packard 5993 gas chromatograph/mass spectrometer (GC/MS) for identification of the separated components.

Figure 26 shows a representative gas chromatogram taken for quality control. The EDMP peak has a retention time of approximately 10.8 minutes. The concentrations of all components are calculated as percents of total peak area.

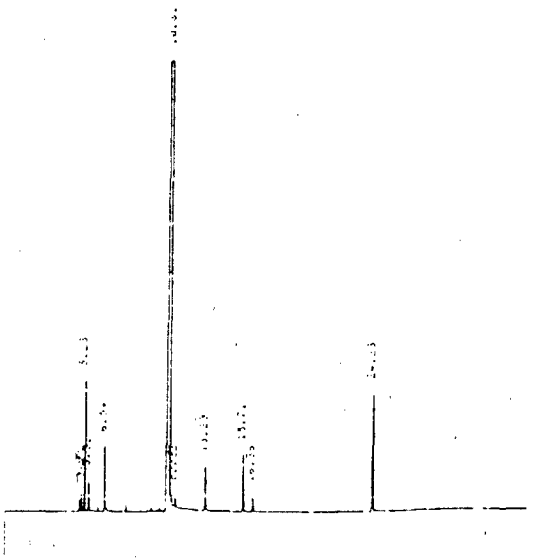


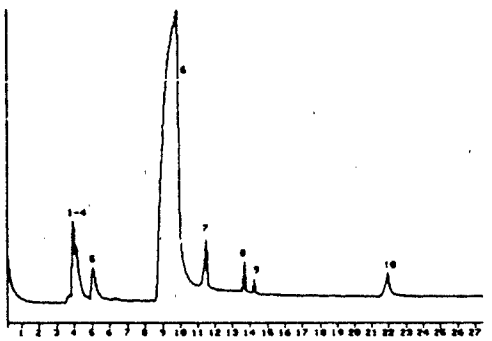
Figure 26. Typical gas chromatogram of EDMP bottle sample.

The EDMP used in the various toxicity studies was stored in 100 ml brown glass bottles. The bottles were equipped with teflon-faced, septum screw caps. This allowed removing a syringe sample from the bottle without removing the cap. The bottles were filled by pressurizing the drums with ultra high purity nitrogen which was also used to purge the headspace above the EDMP in the drums and in the bottles.

Analysis of EDMP immediately after sampling from one of the two drums of bulk material supplied by the Army indicated that purity was about 97%. Analysis after sampling from bottles which had been stored for 13 days showed a 3% drop in purity. A purity of 93% was set as the limit below which the EDMP was considered unfit for use in toxicity experiments. Bottles were rejected only rarely for low EDMP purity due to EDMP breakdown. Experience showed that once a bottle septum was punctured, enough moisture was introduced to cause slow decomposition. Therefore, after the problem had been identified, all bottles of EDMP were discarded if not used on the same day.

A liquid sample of EDMP was injected into the GC/MS using the SE-54 capillary column. Where possible, identifications were made by analysis of the mass spectra and assignment of mass numbers to

molecular fragments. Most of the impurities in the sample were identified as materials detected by Grula and Armelie. They eluted in the same sequence reported by those authors. Although it was not possible to identify the last two impurity peaks from their mass spectra, tentative assignments were made on the assumption that their retention sequence was the same as that described by Grula and Armelie. Figures 27 and 28 are chromatograms of EDMP taken under different conditions to reveal all the impurities. The only peak found in our study which was not seen by Grula and Armelie was diisopropylamine, a very minor impurity in our sample of EDMP.



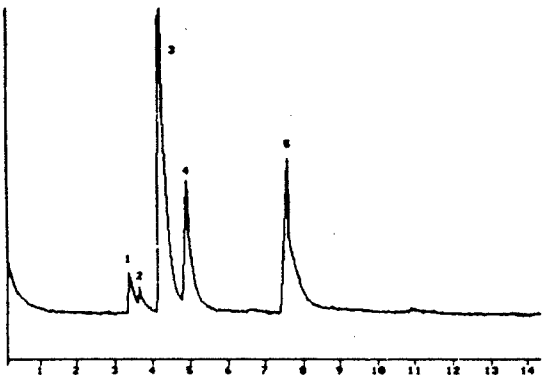
1. ETHANOL
2. DIISOPROPYLAMINE
3. O,O'-DIETHYLMETHYLPHOSPHONITE
4. O-ETHYLMETHYLPHOSPHINATE

} FURTHER RESOLVED

5. DIISOPROPYLAMINOETHANOL
6. EDMP
7. O-(DIISOPROPYLAMINOETHYL) METHYLPHOSPHINATE
8. O-ETHYL,O'-(DIISOPROPYLAMINOETHYL) METHYLPHOSPHONATE
9. Mixture of:
   O-(DIISOPROPYLAMINOETHYL) ETHYLMETHYLPHOSPHINATE*
   O'ETHYL (DIISOPROPYLAMINOETHYL) METHYLPHOSPHINATE*
10. O,O'-BIS (DIISOPROPYLAMINOETHYL) METHYLPHOSPHONITE*

*Assignments made assuming sequence of Grula and Armelie.

Figure 27. Total ion chromatogram of EDMP.



1. ETHANOL
2. DIISOPROPYLAMINE
3. O,O'-DIETHYLMETHYLPHOSPHONITE
4. O-ETHYLMETHYLPHOSPHINATE
5. DIISOPROPYLAMINOETHANOL

Figure 28. Total ion chromatogram of EDMP, gas chromatographic conditions altered to resolve final four peaks.

With the individual decomposition products of EDMP identified, we were able to proceed to the investigation of the stability of EDMP under various chamber conditions.

INVESTIGATION OF THE STABILITY OF O-ETHYL-O'-(2 DIISOPROPYLAMINOETHYL)METHYLPHOSPHONITE

Before acute inhalation exposures with O-ethyl-O'-(2 diisopropylaminoethyl)methylphosphonite could be conducted, it was necessary to characterize the reactions of the compound aerosolized into a chamber as completely as possible both qualitatively and kinetically. This kind of analysis would minimize the chance that unknown reactions might introduce uncertainties into the interpretation of toxicity studies. The experiments were designed to measure the rate of degradation of EDMP under varying conditions of relative humidity

(RH) in nitrogen and air. Once chamber concentration was established by flowing the aerosol through the chamber for 3 minutes at one air change per minute, flow was stopped and samples were removed for analysis to measure the loss of EDMP and formation of products. Products obtained under these conditions were identified and the relative amounts formed were measured. The data obtained from these experiments permitted prediction of the course of chemical change in the exposure chamber. Following the determination of degradation rates, analyses of EDMP and its products was carried out in chambers to be used for inhalation exposure of animals.

In order to perform this investigation, an apparatus was built using a 30 liter glass bell jar as a test chamber and Collison six port nebulizer as an aerosol generator. The system was designed to dilute the EDMP aerosol with air or nitrogen of differing relative humidities. It was planned to attain RH values of 90%, 76%, 52%, or 28% by bubbling the atmosphere through pure water and saturated solutions of potassium bromide (KBr), sodium bromide (NaBr) or calcium chloride ($CaCl_2$) before addition of the concentrated aerosol. Actual values measured by wet and dry bulb psychrometer were approximately 80, 60, 40, and 20% RH. The generation, dilution, and chamber system is shown schematically in Figure 29.

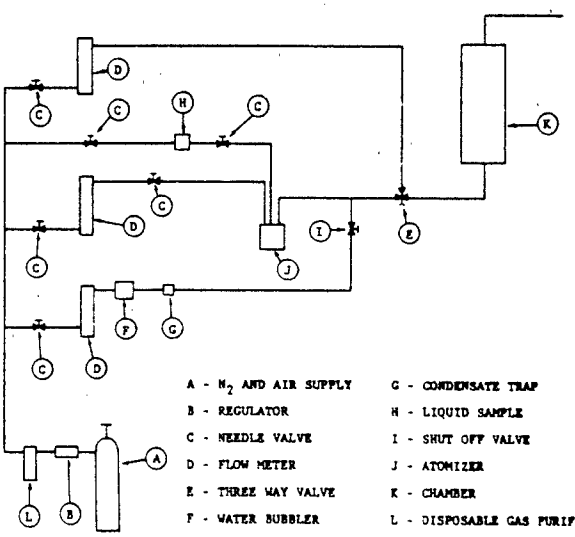


Figure 29. Diagram of system for measuring decomposition rates of EDMP in nitrogen and air.

The flows of the different dilution gases and aerosol generating gases were measured using Fischer Porter flowmeters. The test chamber atmosphere was sampled by passing it through midget impingers containing a solvent, initially hexane, subsequently ethyl ether.

1 µl aliquots of the solvent were then analyzed by gas chromatography and concentrations estimated as peak area/total area. Figure 30 shows a typical chromatograph along with conditions used. The EDMP peak eluted around 18.0 minutes (± 0.2 minutes). The first large peak is the solvent peak.

The first stability test involved generating the EDMP aerosol into an atmosphere of ultra high purity dry nitrogen. In this experiment, the chamber contents were stable over 30 minutes and consisted of about 80% EDMP. The other major component detected in the chamber was O-O'-diethylmethylphosphonite, indicating that the EDMP had disproportionated in the following way:

$$\underset{\text{EDMP}}{CH_3P(O)(OC_2H_5)OCH_2CH_2N(C_3H_7)_2} \longrightarrow \underset{\text{A}}{CH_3P(OC_2H_5)} + \underset{\text{B}}{CH_3P\left[OCH_2CH_2N(C_3H_7)_2\right]_2}$$

The concentrations of the disproportionation products were the same as in dry N_2, indicating that this breakdown reaction occurred in the aerosolizer. The rate of the hydrolysis reaction did not appear to change with relative humidity until 80% RH was reached when all components disappeared rapidly.

With decomposition pathways and rates characterized in nitrogen, a series of experiments was run under static conditions in air. The same disproportionation and hydrolysis reactions occurred as in nitrogen with no evidence of oxidation or any other reaction different from those in nitrogen. Figure 31 is a plot of decomposition under static conditions of EDMP in aerosols produced in nitrogen and air at RH values of 20-60%.

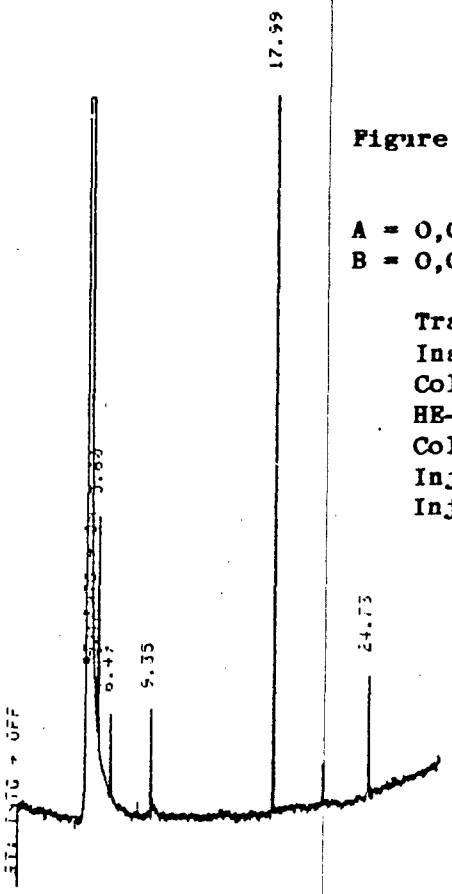
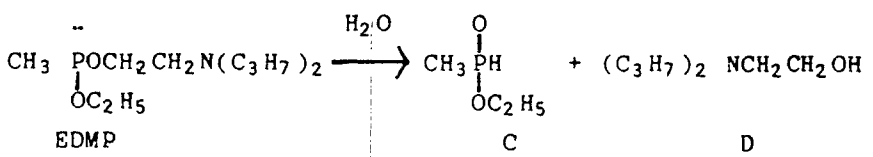


Figure 30. EDMP stability study.

A = O,O'-diethylmethylphosphonite
B = O,O'-bisdiisopropylaminomethylphosphonite

Trace Analysis Solvent Hexane
Instrument: Varian 3700
Column: 50 meter WCOT
HE-28 psi Back Pressure
Column Temperature: 80-230°C at 4 c/min
Injection Size: 1.0 µl Split 90/1
Injection: -220°C DET (FID) 250°C

A series of tests was then performed using ultra high purity nitrogen at relative humidities of 18, 25, 30, and 58%. The results of these tests were similar in that the first sample taken showed an EDMP concentration of 30-70%. Later samples taken showed a drop of EDMP concentration to below 10% in 10-15 minutes. Two major hydrolysis products were identified in the chromatogram representing the following reaction:



EDMP C D

C = O-ethylmethylphosphinate
D = diisopropylaminoethanol

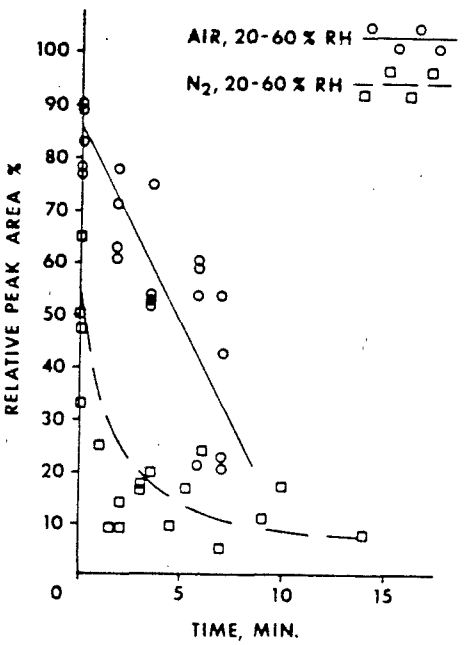
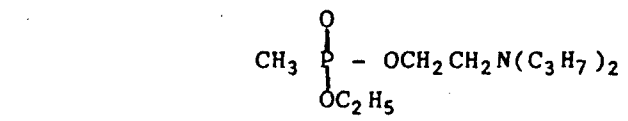


Figure 31. Decomposition of EDMP in aerosols produced in air and nitrogen under static conditions.

The decomposition rate is slightly smaller in air probably because the aerosol introduction line in the air experiments was modified to eliminate a right angle bend. Without impingement at this point, the particle sizes of the aerosols introduced into the chamber during the experiments conducted with air were probably larger than those obtained in the nitrogen experiments leading to slightly lower decomposition rates.

After the experiments in air were concluded, we considered that sufficient information had been obtained on the decomposition pathways and rates to begin measurements in the exposure chamber to be used in the acute exposures of animals to EDMP aerosol. In the first exposure chamber experiment, the sample taken 10 minutes after initial introduction had a relatively high EDMP peak (46% of total). This peak then decreased over the next 4 samples taken

every 30 minutes until it disappeared in the last sample. Concurrently with its disappearance the peak at 24.7 minutes R.T. corresponding to O-ethyl-O'-(diisopropylaminoethyl)methylphosphonate



increased in relative area. A different grade of ethyl ether (Chromatographic rather than "Spectro") had been used in the impingers for collection of these samples. When pure EDMP was dissolved in this ether and allowed to stand, the peak at 24.7 minutes R.T. also gradually replaced the EDMP. Since this compound is an oxidation product of EDMP, it is probable that the EDMP was being oxidized by peroxide present in the ether even though the peroxide concentration is listed on the label as under 0.0001% or 1 ppm. Using the "Spectro" grade ether prevented the appearance of the oxidation peak.

Another chamber experiment was run the next day. This resulted in considerable condensation and/or deposition of liquid on the walls of the chamber. Gas chromatographic analysis of chamber samples showed complete loss of EDMP and very low concentrations of hydrolysis products. Investigation revealed that the chamber humidity control system had malfunctioned and that chamber RH had risen to 78%. Repair of the system reestablished RH at 50% for subsequent experiments.

During the experiments in the exposure chambers, particle size distribution was measured using a Royco® Model 227 counter. Aerosol mass median diameter varied between 2 and 3 μm in these studies.

Following repair of the relative humidity control system for the Rochester chambers, a series of experiments was run to investigate the recovery of EDMP introduced into the chamber by trapping EDMP in ether in an impinger sampler. Another objective was to determine the nature of the material which appeared to condense on the walls of the chamber during introduction of EDMP. The condensate appeared to have low volatility since it remained on the walls even after an overnight purge of the chamber. Consumption of liquid EDMP was measured at 480 mg/min during introduction of the aerosol into the chamber for 50 minutes. The theoretical chamber concentration with a chamber air flow of 10 cfm corresponding to this introduction rate was 1700 mg/m^3. EDMP was introduced into the chamber on four separate days and concentrations were measured after collection in impingers. Average concentrations measured each day were

499, 460, 454, and 408 mg/m^3. The mean chamber concentration for these runs was 455 mg/m^3 which indicated a 27% recovery. Obviously, a satisfactory material balance was not being achieved.

One possible explanation for the low recovery might be that a non-volatile polymerization product was being formed in the chamber and that this product was not detectable by gas chromatographic technique. In order to test this hypothesis, an ethyl ether impinger sample of the chamber atmosphere was evaporated to give an odorless, apparently non-volatile, liquid residue. Ethyl ether was added to the residue and the solution chromatographed. The peak, representing 85% of the chromatogram, was that of diisopropylaminoethanol, one of the hydrolysis products of EDMP. A sample of the condensate on the chamber walls was also chromatographed and yielded almost pure diisopropylaminoethanol. It appears that there is no polymerization of EDMP or its breakdown products, but instead that diisopropylaminoethanol has such a low vapor pressure that it remains on the walls of the chamber after purging. Therefore, the wall condensate cannot be responsible for the low recovery of chamber samples since it is easily measured gas chromatographically.

Another possible cause of the low recovery of material from the chamber was that most of it was lost in the sample lines leading to an impinger placed outside the chamber. In the next series of experiments, the impingers were placed inside the chamber for sampling so that no error of this type was possible.

Because previous work had indicated that the acute inhalation toxicity of EDMP was low, planning of future 6-hour experiments required the ability to introduce high concentrations into the exposure chamber. These concentrations, greater than 2000 mg/m^3, could not be attained by the Collison aerosolizer utilized to this point in the investigation. Therefore, Solo-Sphere nebulizers were obtained for evaluation. These commercial instruments are based on the Babington principle which permits the generation of high concentrations of aerosol with acceptable mass median aerodynamic diameters (MMAD) and geometric standard deviations (σ_g).

In the first experiment with the Solo-Sphere nebulizers, measurement of EDMP volume before and after the introduction period yielded a nominal concentration of 7100 mg/m^3. Concentrations measured by gas chromatographic analysis of 5 impinger samples taken within the chamber over a period of 50 minutes gave an average value of 4900 mg/m^3 with a relative standard deviation of 2.7%. The 69% recovery achieved in this experiment was a great improvement over the 27% previously obtained, demonstrating that the new system of introduction and analysis was superior to the old.

Another experiment was conducted in which samples were obtained on 0.45 μm Teflon filters and separately on three impingers. Filter holders and impingers were placed inside the chamber during sampling. Chamber atmosphere was also directed through an Andersen one cfm sampler to characterize the particle size distribution of the aerosol. The nominal chamber concentration calculated from EDMP consumed and chamber air flow was 6000 mg/m^3. Analyzed concentration from impinger samples was 4000 mg/m^3, to give a recovery of 66%.

Filter samples were eluted in ethyl ether and analyzed by GC. The aerosol concentration thus measured was 1610 mg/m^3. The aerosol concentration calculated by summation of weights on all the stages of the Andersen sampler yielded a value of 1350 mg/m^3, very close to that obtained by filtration. The MMAD derived from sampler weights was 3.6 μm with a σ_g of 1.9, an acceptable value for an aerosol of such high concentration.

These experiments showed that the desired concentration of EDMP with acceptable aerosol particle size can be attained in the chamber to be used for acute studies. A satisfactory material balance was achieved given the uncertainty of air flow measurement. The only task remaining to prepare for acute inhalation studies is the development of continuous analysis procedures for chamber concentration.

CHEMICAL ANALYSIS OF THE HYDRAULIC FLUIDS, DURAD MP280, AND FYRQUEL 220

The Toxic Hazards Research Unit (THRU) of the Department of Community and Environmental Medicine, University of California, Irvine carried out experiments to characterize the toxicity of the triarylphosphate hydraulic fluids, Durad MP280, and Fyrquel 220. Experiments performed included irritation and sensitization tests; acute oral, dermal and inhalation exposures; delayed neurotoxicity tests using chickens as the test species; and two-week intermittent and 90-day continuous inhalation exposures. Results of the neurotoxicity experiments showed that Durad MP280 was a delayed neurotoxin to chickens with a TOCP equivalent of 31% (MacEwen and Vernot, 1982) while Fyrquel 220 had no adverse effects in this species.

Because of the positive neurotoxicity findings using Durad MP280, NMRI/TD requested that the o-cresol content of this fluid and Fyrquel 220 be determined using the technique outlined in MIL-H-19457C(SH). This military specification for triarylphosphate

hydraulic fluids stipulates that the triorthocresylphosphate (TOCP) content of the fluid shall not be greater than one percent, apparently in the belief that this will insure that the fluid is non-neurotoxic. However, the analytical method designated in the military specification for hydraulic fluids is not specific for TOCP and does not directly measure TOCP. Instead, it measures o-cresol after hydrolysis of the phosphate esters. At low concentrations most o-cresol containing phosphates will be present as monosubstituted compounds rather than as TOCP. Nevertheless, one can be certain that TOCP concentration in the fluid will be less than that of o-cresol in the hydrolyzate, and, in that sense, o-cresol content is an upper limit for possible TOCP concentration in the hydraulic fluid.

In response to the request by NMRI/TD, the THRU analyzed hydrolyzates of Durad MP280 and Fyrquel 220 for o-cresol content essentially by the method given in MIL-H-1945C(SH) summarized below:

A sample of the phosphate ester is saponified overnight in a Parr bomb containing aqueous caustic solution. After neutralization, the phenolic fraction is extracted with diethyl ether. The ether solution is chromatographed and o-cresol is determined after standardization using known mixtures of o-cresol in diethyl ether.

Subsequently, NMRI/TD asked that the individual phenols in the hydrolyzates be identified in an effort to distinguish the specific elements in Durad MP280 responsible for its neurotoxicity. Gas chromatograph/mass spectrometric (GC/MS) analysis was performed on the hydrolyzates to implement this request.

The calibration data for quantitative analysis of o-cresol by the method given in MIL-H-19457C(SH) are listed in Table 89.

The regression equation relating concentration to peak area was calculated using the information in the table.

$$\% \text{ o-Cresol in Hydrolyzate} = \text{Peak Area} \times 2.31 \times 10^{-6} - 0.001$$

The correlation coefficient of this calibration equation is 0.99999.

Duplicate samples of Durad MP280 and Fyrquel 220 were hydrolyzed, extracted and analyzed for o-cresol. The peak areas and calculated o-cresol concentrations are presented in Table 90.

TABLE 89. GAS CHROMATOGRAPHIC PEAK AREA MEAN VALUES OBTAINED FROM O-CRESOL STANDARDS

% o-Cresol[a]	Number of Samples	Mean Peak Area, G.C. Units
0.02	3	8,932
0.15	3	66,403
0.20	18	86,264
0.40	3	174,550
1.00	6	434,150

[a]Calculated concentration of o-cresol in the hydraulic fluid.

TABLE 90. O-CRESOL CONCENTRATIONS IN HYDROLYZATES OF DURAD MP280 AND FYRQUEL 220

Sample	Peak Area, Units	% o-Cresol
DURAD MP280		
Hydrolyzate 1	70,800	0.163
Hydrolyzate 2	73,310	0.168
Mean		0.166
FYRQUEL 220		
Hydrolyzate 1	7,875	0.017
Hydrolyzate 2	7,437	0.016
Mean		0.017

Analyses of the two hydraulic fluid hydrolyzates using the GC/MS gave the total ion chromatograms and identifications shown in Figure 32 for Durad MP280 and Figure 33 for Fyrquel 220.

All specific isomers were identified except the isomeric xylenols which have spectra so similar they cannot be easily distinguished and the isomeric methyl ethyl phenols, several of which give very similar mass spectra.

The area percentages of the components were taken from the total ion chromatographs and are shown for Durad MP280 and Fyrquel 220 in Tables 91 and 92, respectively. The percentages shown cannot be interpreted as precise weight percentages in the hydraulic fluids because accuracy and precision are poor for low concentration constituents, and incomplete peak resolution for high concentration components makes quantitative determination difficult.

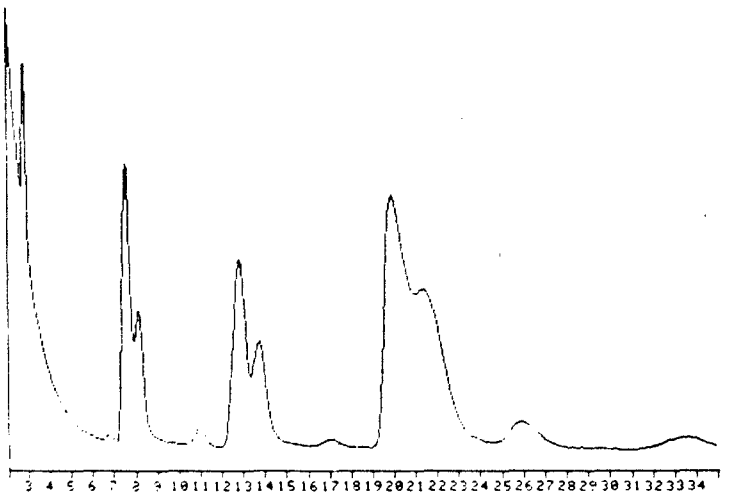


Figure 32. Total ionization gas chromatogram of the phenols in hydrolyzed Durad MP280.

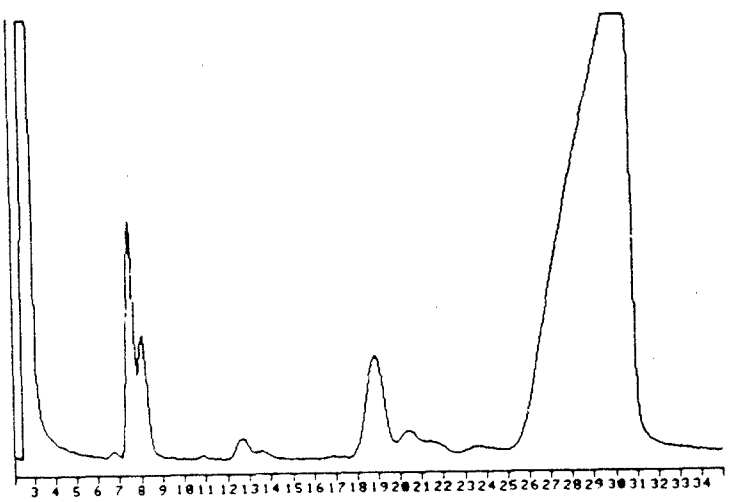


Figure 33. Total ionization gas chromatogram of the phenols in hydrolyzed Pyrquel 220.

TABLE 91. GAS CHROMATOGRAPHIC IDENTIFICATION OF PHENOLS HYDROLYZED FROM DURAD MP280

Compound	Retention Time, Min.	Peak Area, %
Phenol	3.0	2.4
o-Methylphenol	6.8	0.2
m-Methylphenol	7.6	13.6
p-Methylphenol	8.8	4.2
o-Ethylphenol	11.1	0.9
m-Ethylphenol	12.9	14.2
p-Ethylphenol	14.6	6.1
Xylenol	17.0	0.6
Xylenol	20.3	40.0
Xylenol	23.1	12.2
Xylenol	26.2	3.2
Methylethylphenol	33.2	2.5

TABLE 92. GAS CHROMATOGRAPHIC IDENTIFICATION OF PHENOLS HYDROLYZED FROM FYRQUEL 220

Compound	Retention Time, Min.	Peak Area, %
Phenol	4.3	24.5
o-Methylphenol	6.8	0.1
m-Methylphenol	7.6	5.3
p-Methylphenol	8.6	1.1
o-Ethylphenol	11.0	0.1
m-Ethylphenol	12.7	0.6
p-Ethylphenol	14.0	0.2
Xylenol	18.8	4.1
Xylenol	20.6	1.1
Xylenol	22.0	0.3
Xylenol	23.7	0.2
Methylethylphenol	29.3	62.5

Nonetheless, Fyrquel 220 is the simpler mixture since 87% is represented by phenol and p-t-butylphenol. Durad MP280, however, has significant concentrations of methylphenols, ethylphenols, and xylenols.

Analyses of o-cresol in Durad MP280 and Fyrquel 220 hydrolyzates established that both hydraulic fluids comply with MIL-H-19457C(SH), but that Durad MP280 has ten times more o-cresol than Fyrquel 220. They also provide evidence that the methods and stipulations given in that military specification do not guarantee against neurotoxicity of the fluid as measured by the chicken test.

Previous investigators (Bondy et al., 1960; Johannsen et al., 1977) have demonstrated that delayed neurotoxicity in triarylphosphates is associated with ortho-alkyl substitution and, further, that the presence of hydrogen on the alpha carbon is required for activity. Moreover, mixed phosphate esters containing one active phenol group are more toxic than disubstituted compounds which, in turn, have greater toxicity than symmetrical active esters. Dialkyl substitution in the phenolic portion of the ester appears to negate the toxic potential since Bondy et al. (1960) were able to produce a non-neurotoxic mixture of trixylenylphosphates by removing o-cresol, o-ethylphenol and o-propylphenol from the raw material used for esterification. One phosphate ester which did not obey any of the empirical rules set up for structure-activity relationships was tri-p-ethylphenyl phosphate which was extremely neurotoxic while mono- and di-substituted p-ethylphenylphosphate did not possess toxicity.

Because the triaryl phosphates are synthesized from phenolic mixtures, the final distribution of each phenol among the esters should be a statistical function of its initial concentration. Since the phenol may participate in mono-, di- or tri-substitutions, one can calculate the probability of each category as a binomial raised to the third power or:

$$(A + B)^3 = A^3 + 3A^2B + 3AB^2 + B^3$$

A = Concentration of active phenol in initial mixture
B = $1 - A$
A^3 = Concentration of trisubstituted ester
$3A^2B$ = Concentration of disubstituted ester
$3AB^2$ = Concentration of monosubstituted ester
B^3 = Concentration of rest of ester mixture

Using these relations, the concentrations in Durad MP280 and Fyrquel 220 of the putatively most toxic esters, i.e. the monosubstituted ortho esters and tri-p-ethylphenylphosphate, were calculated from GC and GC/MS hydrolyzate data and are listed in Table 93.

TABLE 93. CALCULATED CONCENTRATIONS OF NEUROTOXIC ESTERS IN DURAD MP280 AND FYRQUEL 220

Compound Type	Concentration, (%) Durad MP280	Fyrquel 220
Mono-o-cresyl phosphate	0.4	0.04
Mono-o-ethylphenylphosphate	2.6	0.3
Tri-p-ethylphenylphosphate	0.02	8×10^{-7}

Even considering the uncertainty introduced by using GC/MS concentrations for the ethylphenols in the hydrolyzates, Durad MP280 has an order of magnitude greater concentration of neurotoxic esters than Fyrquel 220. The calculations also reveal a weakness in the requirements outlined in MIL-H-1945C(SH) which stipulates that the TOCP concentration of the fluid should not be greater than one percent. Since low concentrations of o-cresol would lead to negligible concentrations of TOCP but possibly significant concentrations of mono-o-cresyl phosphate and other neurotoxic esters, the specification does not focus on the components with the highest toxic potentials. Triarylphosphate hydraulic fluids which meet this requirement of MIL-H-1945C(SH) may still possess substantial neurotoxic potential as measured by administration to chickens. If chemical constitution is used to define acceptability of an hydraulic fluid, it may be desirable to limit the sum of all known neurotoxic phenolic constituents in the hydrolyzate to a value leading to a maximum acceptable concentration of neurotoxic esters. Alternatively, delayed neurotoxicity may be estimated for all triarylphosphate fluids by means of the chicken test.

GAS CHROMATOGRAPHIC/MASS SPECTROMETRIC (GC/MS) ANALYSIS OF PETROLEUM JP-8 JET FUEL

In order to provide a chemical characterization of petroleum JP-8 exposure, a sample of the 1000 mg/m^3 chamber atmosphere was chromatographed in the GC/MS to give the total ion chromatogram in Figures 34 and 35. The concentration was too small to identify any but the highest peaks. Therefore, a liquid sample of JP-8 was injected into the instrument and 28 components identified. The chromatograms of liquid JP-8 are given in Figures 36 and 37 and the identified components in Table 94. Peaks in the chamber atmosphere sample with the same retention times as those in the liquid sample were given those identifications and are so noted in the Figures.

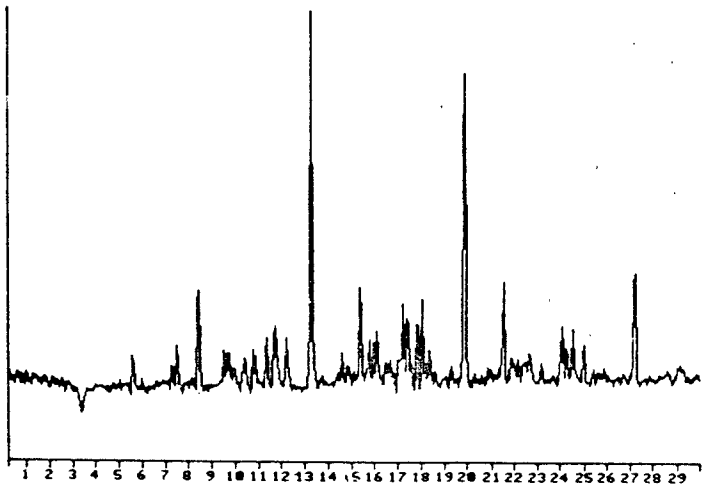


Figure 34. Total ion chromatogram of chamber JP-8 - more volatile peaks.

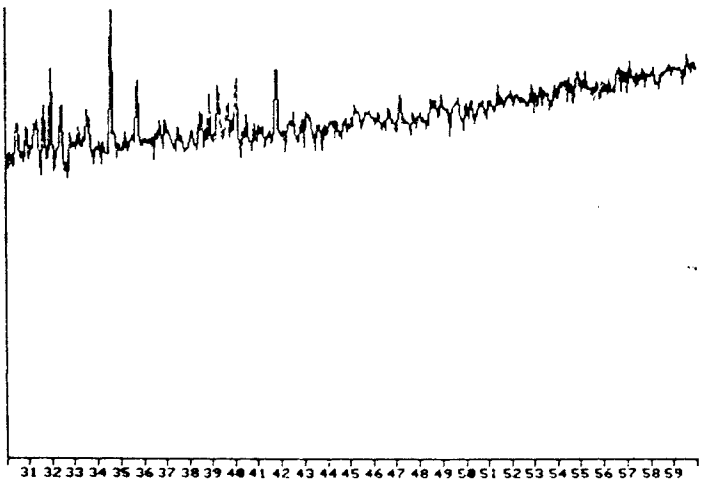


Figure 35. Total ion chromatogram of chamber JP-8 - less volatile peaks.

As expected, the compositions of the two samples are similar; the major differences being a shift to higher concentration of the more volatile constituents in the chamber atmosphere sample.

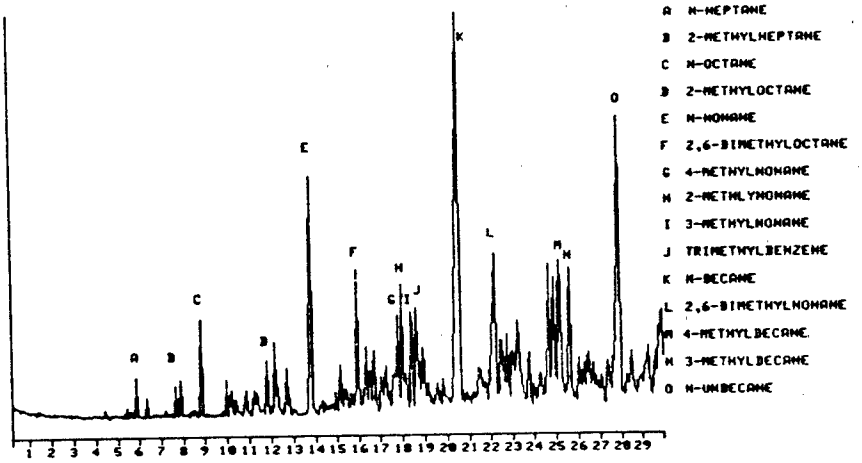


Figure 36. Total ion gas chromatogram of liquid JP-8 - more volatile peaks.

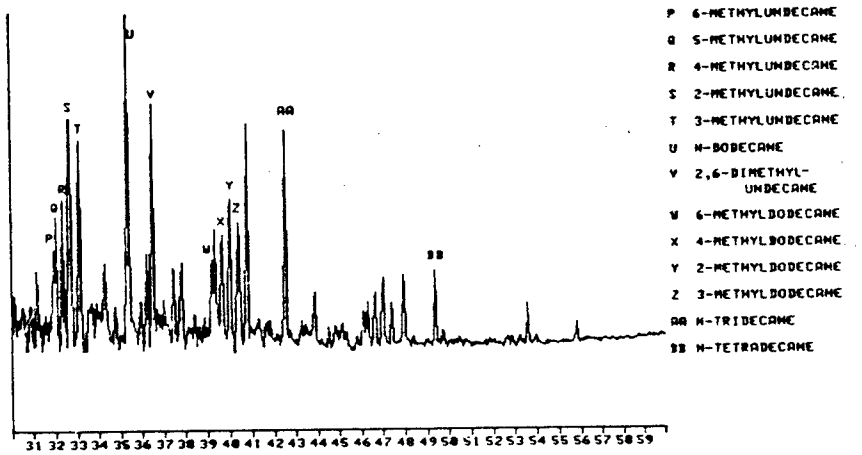


Figure 37. Total ion gas chromatogram of liquid JP-8 - less volatile peaks.

TABLE 94. COMPONENT IDENTIFICATION IN CHROMATOGRAMS OF LIQUID AND CHAMBER ATMOSPHERE JP-8

A	N-HEPTANE	O	N-UNDECANE
B	2-METHYLHEPTANE	P	6-METHYLUNDECANE
C	N-OCTANE	Q	5-METHYLUNDECANE
D	2-METHYLOCTANE	R	1-METHYLUNDECANE
E	N-NONANE	S	2-METHYLUNDECANE
F	2,6-DIMETHYLOCTANE	T	3-METHYLUNDECANE
G	4-METHYLNONANE	U	N-DODECANE
H	2-METHYLNONANE	V	2,6-DIMETHYLUNDECANE
I	3-METHYLNONANE	W	6-METHYLDODECANE
J	TRIMETHYLBENZENE	X	4-METHYLDODECANE
K	N-DECANE	Y	2-METHYLDODECANE
L	2,6-DIMETHYLNONANE	Z	3-METHYLDODECANE
M	4-METHYLDECANE	AA	N-TRIDECANE
N	3-METHYLDECANE	BB	N-TETRADECANE

THOMAS DOME INHALATION EXPOSURE CHAMBER NOISE ABATEMENT PROGRAM

Noise intensity measurements made in previous years in the inhalation exposure chambers have indicated that the OSHA limits for human exposure have never been exceeded. However, in an effect to minimize discomfort to chamber technicians servicing the domes and to reduce stress on laboratory animals, a program to reduce noise levels in the domes was begun and implemented during the year.

Preliminary examination indicated that the most significant contributors to high noise levels in the domes were the input control valves. These are diaphragm valves in which the flowing air has to move through two 90 degree bends. This seemed to be the point at which most of the noise was generated.

A series of tests was designed to evaluate the results of a number of changes or additions to the input air system using Chamber No. 1 as the test chamber. Readings of sound intensity were taken using a Bruel and Kjaer Model No. 2209 precision sound level meter equipped with a Model No. 1613 octave band filter set and a Model No. 4265 free field microphone. The microphone was located at the chamber center which was identified as the peak noise location and two meters above the floor for all readings taken. Vacuum Pump A-3 was used to maintain the chamber at 5 mmHg negative pressure differential.

Two approaches were taken to alternate the sound level in the exposure chamber. The first was replacement of the valve with one which generated a minimum of noise, and the second was the insertion of a spool piece containing foam covered baffles to absorb the sound generated in the valve. The THRU Engineering group obtained a number of different types of valves and constructed baffles of different designs for testing. Valves were tested singly to determine the most effective changes. Then the optimum valve type was combined with the various baffles, and the noise intensity in the exposure chamber measured with the combination in place. The various valve substitutions and additions tested are given in Table 95.

TABLE 95. MODIFICATIONS TO EXPOSURE CHAMBER NO. 1 INPUT AIR SYSTEM TESTED FOR NOISE REDUCTION

1. Straight-through diaphragm input control valve.
2. Butterfly input control valve replacing spool piece.
3. 3-baffle muffler in input air line downstream from butterfly input control valve.
4. Stainless steel input air line replaced with PVC plastic pipe.
5. 6-baffle muffler replacing 3-baffle style downstream from butterfly input control valve.
6. Large bore 6-baffle muffler replacing original 6-baffle muffler downstream from butterfly input control valve.

The most effective reduction of noise levels was accomplished with the butterfly input control valve and the large bore 6-baffle muffler. Figures 38 and 39 compare the noise intensity spectra of this combination with this original configuration at 20 and 60 cfm chamber air flow rates. Peak levels are reduced about 15 decibels and shifted out of this speech range from 2000 Hz to 250 Hz.

Figure 40 shows the effect of this modification on A-weighted sound level. A-weighting is a method of combining the contributions of noise at all frequencies to estimate an overall hearing sound level. The plots in Figure 40 demonstrate that the change has decreased the A-weighted sound level by more than 20 decibels over all chamber air flow rates tested.

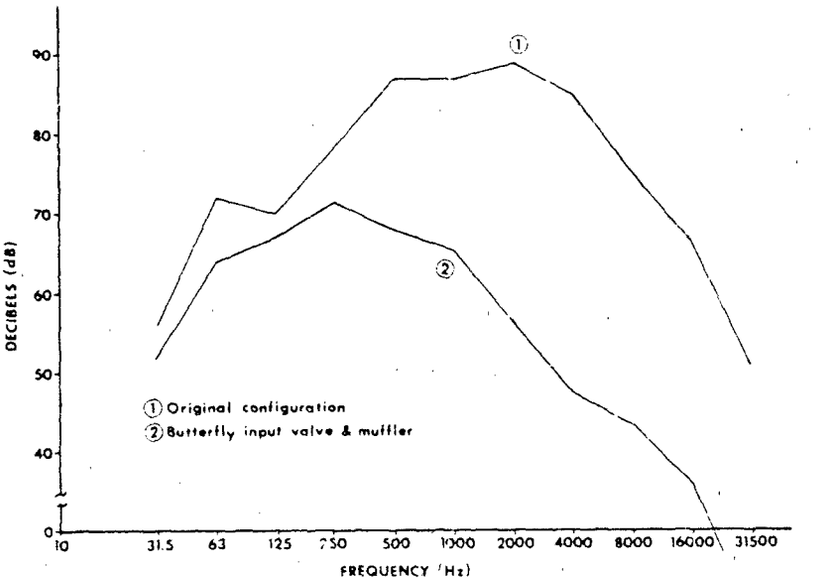


Figure 38. Frequency spectra of sound level at 20 cfm chamber air flow.

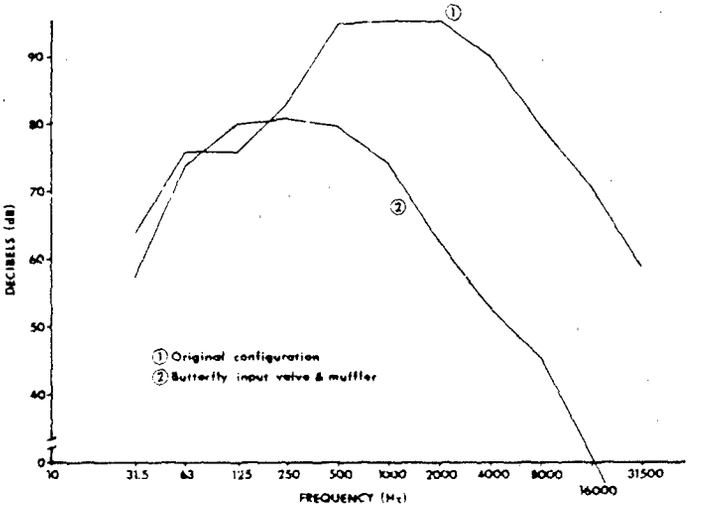


Figure 39. Frequency spectra of sound level at 60 cfm chamber air flow.

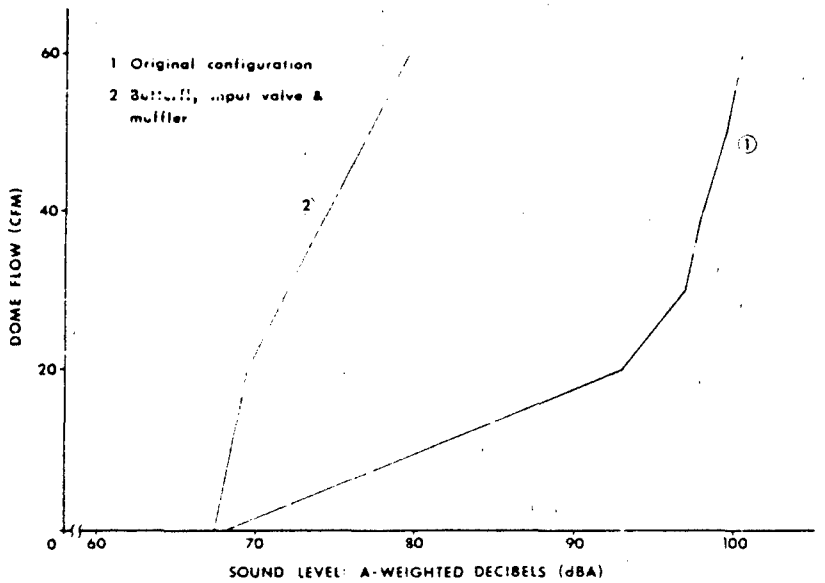


Figure 40. Weighted sound levels at different chamber air flows.

Four of the Thomas Dome exposure chambers have been modified to the new configuration and the other four are in the process of modification.

DEPOSITION AND CLEARANCE OF RADIOLABELED PARTICLES INHALED DURING CO_2 EXPOSURES

A number of mechanical, phagocytic and biochemical defense mechanisms help provide protection from inhaled particles (Killough, 1979). Among these, mucociliary transport removes those particles deposited on the mucus of the nose, pharynx, and bronchial tree. Among the several mechanisms for deep lung clearance is engulfment and transport by pulmonary macrophages. Since these clearance mechanisms perform very essential roles in the defense of the whole animal, a test of their efficacy is of interest when evaluating the toxicity of potential airborne agents. Previous investigators have shown pulmonary clearance to be altered by exposures to many agents including ozone (Frager et al., 1981), sulfuric acid mist (Wolff et al., 1981), halothane (Wolff and Muggenburg, 1980), oxygen (Laurenzi et al., 1968; Sackner et al., 1975), carbon dioxide (Marin and Morrow, 1969), and cigarette smoke (Albert et al., 1975; Reznik and

Borgmeyer, 1980; Reznik and Samek, 1980). Since any decrease in pulmonary clearance would presumably lead to increased susceptibility to injury, prolonged retention could, at least in part, be the cause of increased mortality and shortened survival time of rats and mice exposed to streptococci after exposure to nitrogen dioxide and ozone (Coffin et al., 1968; Ehrlick et al., 1979; Goldstein et al., 1974). Similarly, prolonged particle retention could play a role in the association seen in man between smoking and death from lung cancer, chronic bronchitis, and pulmonary tuberculosis (Doll and Hill, 1974).

Although there are several methods of measuring pulmonary clearance, the use of radiolabeled microspheres deposited in the lungs via inhalation permits quantitative assessment of the various clearance mechanisms of the lungs. Such tests have proved to be sensitive in measuring the effects of air pollutants (Frager et al., 1979; Kenoyer et al., 1981). However, this sensitivity requires a large sample size because of the large standard deviation observed in the radioactivity clearance measurements.

One possible source of this variation could be the inhomogeneity of radiolabeled microsphere deposition within the lung. In this study, attempts were made to improve the methodology of particle deposition and clearance measurements in rats. The use of a simultaneous exposure to CO_2 during the inhalation of radiolabeled microspheres might reduce the inhomogeneity of deposition by standardizing ventilation at a higher level and increasing total deposition (Brain and Varberg, 1979). Thoracic radioactivity in previous studies (Frager et al., 1979; Kenoyer et al., 1981; Mannix et al., 1982; Phalen et al., 1980) has been measured using a detector placed above the rat's thorax. This does not intercept most of the thoracic radiation being emitted. Therefore a ring-shaped detector was designed to give 360° detection. Since the resulting higher count/unit time improves the accuracy and precision in the radioactivity measurements and also allows measurements to be made over a longer period of time postexposure, use of the ring detector might also improve test precision and sensitivity.

This study was designed to achieve the following objectives: (1) compare results obtained from exposure to radiolabeled microspheres with and without simultaneous exposure to CO_2, (2) evaluate how accurately external thoracic measurements of radioactivity correspond to direct radioactivity measurements made on excised lungs, (3) evaluate any change in the pattern of particle deposition within the lung produced by the CO_2 exposure, and (4) compare the results

of measuring clearance by thoracic measurements versus measurements of the radioactivity excreted in the feces (Prager et al., 1979; Kenoyer et al., 1981; Mannix et al., 1982; Phalen et al., 1980).

Monodispersed polystyrene latex (PSL) microspheres were labeled with a tightly bound ^{51}Cr isotope (28 day half life, 0.32 MeV gamma emmission) as previously described (Hinrichs et al., 1978). In vitro leaching studies had indicated a leaching rate of ^{51}Cr from the particles of less than 0.1% per day (Hinrichs et al., 1978).

The microsphere exposure system, diagrammed in Figure 41, consisted of an aerosol generator, exposure unit, and containment system. A Lovelace-type compressed-air nebulizer was operated at 40 psig to generate the aerosol. The reservoir contained a 0.1% suspension of ^{51}Cr labelled PSL particles with a total activity of about 5 mCi. The resultant aerosol was characterized using a seven stage cascade impactor and had a mass median diameter of 1.4 μm and a geometric standard deviation of 1.3. After nebulization, the particles were mixed with a diluting air stream and heated to 70°C for drying which limited the agglomerates to less than 5%. Breathing air, used in the nebulizer and for dilution, contained added CO_2 when required for the exposure schedule. In order to neutralize electrostatic charge on the aerosol, the particles were passed through a 1 mCi ^{85}Kr deionizer (TSI, St. Paul, MN). The aerosol was then delivered through a small animal, nose-only exposure unit which may hold up to 48 rats at a time. The total activity within the chamber was approximately 250 μCi. The rats were held in 20 cm long, 5 cm diameter plastic tubes and were exposed for 20 minutes.

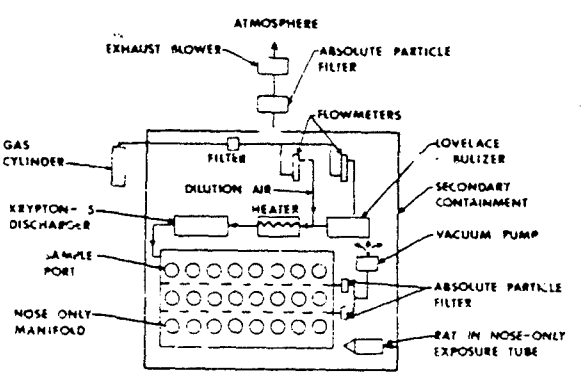


Figure 41. Exposure system for the deposition of ^{51}Cr labeled microspheres in rats.

The aerosol was exhausted through absolute particle filters which entrapped the radiolabeled aerosol for subsequent disposal. The exposure unit was held slightly below ambient pressure to minimize loss of the aerosol through the animal tubes.

The entire exposure unit was located within a secondary containment system with an airflow of 650 CFM and a downstream HEPA filter to entrap any radiolabeled particles which might escape.

External thoracic radioactivity measurements were made by placing the rat in a holding tube and then inserting the tube within the ring shaped NaI(Tl) detector shielded by lead and collimated such that radiation from the respiratory tract was favored for detection (Figure 42). A multichannel analyzer integrated counts in the energy region of interest. All thoracic radioactivity measurements were made for at least 100 seconds to obtain a minimum of 1000 counts. The holding tubes for radioactivity measurements were 25.4 cm long and 6.3 cm in diameter with an adjustable tail gate. The front end of the tubes had 1.3 cm diameter concentric holes into which the rats would voluntarily thrust their noses. This standardized the position of the rats within the tubes for the serial radioactivity measurements. Fecal radioactivity measurements were made using a Na(Tl) well detector.

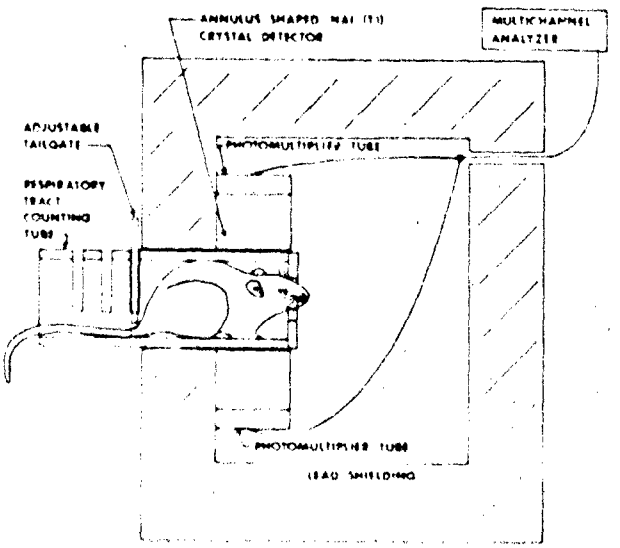


Figure 42. Ring shaped crystal detector for measurement of thoracic radioactivity.

Sprague-Dawley male rats, 175-225 g., free of Mycoplasma pulmonis were used. The animals had food and water ad libitum except during the aerosol exposure and radioactivity measurements. For 12 days prior to the study, the animals were caged in single layer wire bottom Rochester Chambers in order to provide as dust-free a pre-exposure environment as possible. After the aerosol exposures, the animals were caged in individual wire bottom cages.

In order to determine the gross deposition pattern and short-term fate of the radiolabeled particles deposited both internally and externally, the rats were exposed to the particles in air and in 3% and 5% CO_2 for 20 minutes and then killed at intervals over the next 48 hours. The radioactivity of the following four areas was measured: (1) excised trachea and lungs, (2) defurred head after decapitation, (3) gastrointestinal tract and internal organs, and (4) remaining carcass including head fur.

After the 20 minute exposure, the rats were killed using 75 mg/kg phenobarbital i.p., and lungs excised, inflated and dried for 24 hours at an air pressure of 30 cm water. The lobes were separated during drying by pieces of parafilm. After drying, the left lung and the apical, cardiac, diaphragmatic and azygous lobes of the right lung were cut off at the most distal points of their respective bronchi. The left lung was then cut into 12 equal parts on 3 horizontal planes and 4 vertical planes. In a similar manner, the diaphragmatic lobe was cut into 9 equal sections and the other lobes into 4 parts. The 33 pieces thus obtained (Figure 43) and the remaining tracheal/bronchial section were individually weighed and placed in culture tubes for radioactivity measurements. To minimize cross contamination, a new razor blade was used for each rat, and the blade was cleaned prior to each cut. The specific radioactivity and the Evenness Index (E.I.) defined as the ratio of specific activity of each piece to the lung's average specific activity were calculated for each dried lobe section (Brain et al., 1976).

We hypothesized that CO_2 might have two different effects on the clearance of microspheres: 1) it might influence the total deposition and distribution pattern of inhaled microspheres and/or 2) it might affect the clearance of the particles from the lung after deposition. In order to differentiate between these possibilities, an additional experimental protocol was devised which combined 20 minute inhalation of microspheres in atmospheres of different CO_2 concentrations with subsequent 20 minute exposure to air or CO_2 according to the following scheme:

Preexposure Atmosphere	Exposure Atmosphere			
	Air	Air	3% CO_2	5% CO_2
Air	X		X	X
5% CO_2		X		

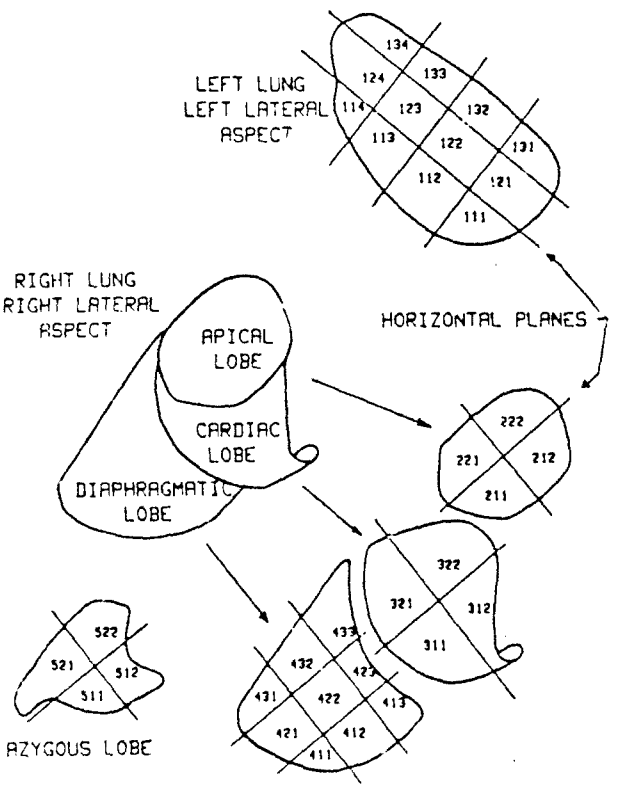


Figure 43. Diagram of lung showing location and numbering of lung sections.

This sequence produced equal tube residence times for all exposures and allowed differentiation between direct CO_2 effects and CO_2 induced alterations in the microsphere deposition pattern. After the exposures the rats were removed from the unit and their heads were wiped with cotton balls to remove particles and to minimize contamination of the counting system. Serial external thoracic radioactivity measurements were made throughout 934 hours postdeposition.

Sixteen fecal collections from each rat were made during the first 118 hours postdeposition of the radiolabeled microspheres to determine the fecal excretion activity curve (Kenoyer et al., 1981). The feces were collected from the trap below the individually caged rats and combined with the feces obtained by placing the rats back into the holding tubes, a procedure which induced defecation.

Residual analysis of the data showed that a 3-component curve of the form $Y = A_1 \exp(-B_1 t) + A_2 \exp(-B_2 t) + A_3 \exp(-B_3 t)$ was needed to fit the lung radioactivity decay curve. The intercept and slope for each component was analyzed using Student's t-test.

The E.I. data from the pattern of particle deposition within the lung were evaluated using a two-way analysis of variance.

The fecal radioactivity data were fitted to a lognormal function and the time at which 50% of the activity excreted throughout 50 hours postdeposition was determined for each rat and analyzed using a Student's t-test. The significance level was set at 0.05.

Table 96 shows the external thoracic radioactivity and the pattern of deposition of the radiolabeled microspheres in the rat immediately after 20 minute deposition exposures in air and 5% CO_2. The exposure to microspheres in 5% CO_2 did increase the total amount of particles deposited by about 40% but did not have a significant effect on the gross pattern of deposition. Of note was the location of 36% of the particles in the gastrointestinal (GI) tract immediately after the 20 minute exposure. Figure 44 shows the change of relative radioactivity with time in animals exposed in normal air. By 1 hour after deposition, 63% of the external thoracic count emanated from particles within the GI tract. Therefore, as shown in Figure 45 where the ratio of the external thoracic to lung radioactivity is plotted versus time, external thoracic radioactivity measurements do not accurately reflect lung radioactivity until 30 hours after deposition. For that reason fecal radioactivity was used to monitor clearance during that time

frame. The times required to clear 50% of the particles in the first component of the excretion curve were 13.7 and 13.5 hours for the air and CO_2 exposed rats, respectively.

TABLE 96. EXTERNAL THORACIC RADIOACTIVITY IMMEDIATELY AFTER DEPOSITION, AND RADIOACTIVITY WITHIN THE RAT AS A PERCENTAGE OF THE EXTERNAL THORACIC COUNT

	Air $\bar{X} \pm$ S.E., (N = 6)	5% CO_2 (N = 3)
External Thoracic (CPM)	6170 ± 769	8658 ± 1160
Lungs-Trachea (%)	23.4 ± 3.5	21.2 ± 2.8
Defurred Head (%)	11.7 ± 2.8	14.9 ± 6.1
GI Tract-Organs (%)	36.1 ± 7.2	24.9 ± 3.3
Carcass-Head Fur (%)	31.2 ± 2.9	39.0 ± 6.3

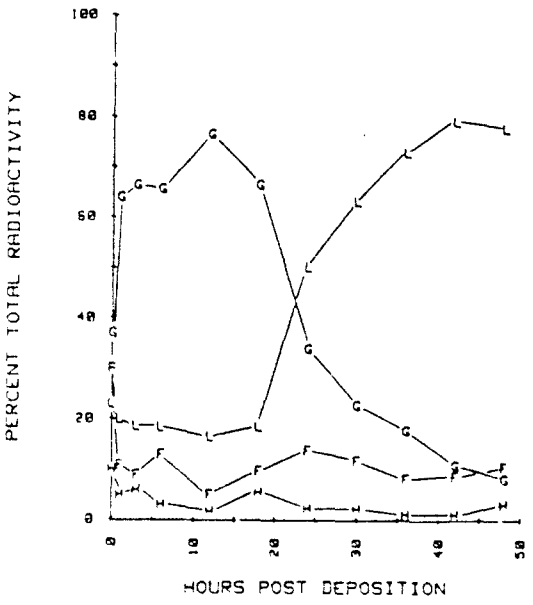


Figure 44. Percent of the total radioactivity deposited and remaining with time in the lungs (L), defurred head (H), gastrointestinal tract and internal organs (G) and on the head fur with remaining carcass (F). Rat exposed to aerosol in air.

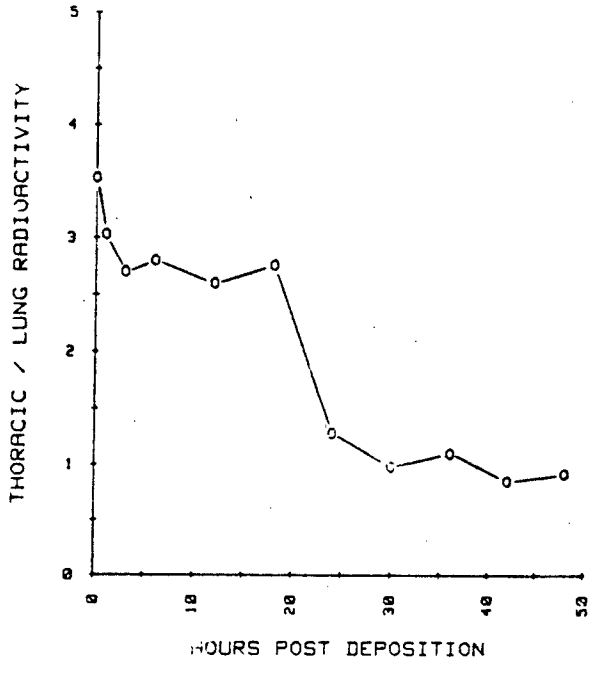


Figure 45. Ratio of external thoracic to excised trachea-lung radioactivity as a function of time after radiolabeled microsphere deposition. Rats exposed to aerosol in air.

Table 97 illustrates that inhalation of the microspheres in 5% CO_2 has led to an increase in lung and tracheal deposition with the increase in the trachea being relatively greater than that in the lungs although tracheal deposition is insignificant compared to that in the lungs.

Tables 98, 99, and 100 present the deposition data within the left lung and right lung lobes after inhaling particles in air or CO_2 mixtures of 3% and 5%. During air breathing, the particles were preferentially deposited to the more apical and dorsal sections of the lung. At elevated inspired CO_2 levels, the difference between the terminal sections is reduced indicative of more uniform distribution within the lobe. However, over the entire lobe the reduction is not statistically significant. In addition, the preferential deposition to the left lung and apical lobe of the right lung was not altered by elevated inspired CO_2 levels.

TABLE 97. EFFECT OF A SIMULTANEOUS EXPOSURE TO CO_2 AND RADIOLABELED MICROSPHERES ON THE DEPOSITION OF MICROSPHERES WITHIN THE RAT LUNG

Counts/Minute/µg tissue; $\bar{X} \pm$ S.E., N = 12

	Air	5% CO_2
Lung	39.69 ± 1.89	49.09 ± 4.55
Trachea	0.19 ± 0.02	0.31 ± 0.06

TABLE 98. EFFECT OF A SIMULTANEOUS EXPOSURE TO CO_2 AND RADIOLABELED MICROSPHERES ON THE DEPOSITION PATTERN OF THE MICROSPHERES WITHIN THE RAT LUNG: HORIZONTAL PLANES

	Horizontal Plane	Evenness Index; $\bar{X} \pm$ S.E., N = 12			Significant ($p < 0.05$)[a]	
		Air	3% CO_2	5% CO_2	CO_2	Plane
Left Lung	131-134	1.31 ± 0.05	1.29 ± 0.08	1.28 ± 0.05		
	121-124	0.81 ± 0.03	0.86 ± 0.05	0.83 ± 0.05		X
	111-114	0.99 ± 0.06	1.08 ± 0.11	1.24 ± 0.12		
Apical Lobe	221-222	1.64 ± 0.08	1.50 ± 0.10	1.55 ± 0.09		X
	211-212	1.16 ± 0.04	1.07 ± 0.08	1.06 ± 0.06		
Cardiac Lobe	321-322	0.96 ± 0.03	0.85 ± 0.06	0.89 ± 0.04	X	
	311-312	0.95 ± 0.03	0.78 ± 0.03	0.88 ± 0.05		
Diaphragmatic Lobe	431-433	1.25 ± 0.05	1.08 ± 0.08	1.03 ± 0.06		
	421-423	0.75 ± 0.03	0.75 ± 0.04	0.73 ± 0.04		X
	411-413	0.80 ± 0.06	1.02 ± 0.10	0.89 ± 0.03		
Azygous Lobe	521-522	0.72 ± 0.05	0.80 ± 0.09	0.89 ± 0.06		
	511-512	0.58 ± 0.07	0.69 ± 0.05	0.65 ± 0.09		

[a] 2-way ANOVA

Figure 46 is a plot of the decay of the external thoracic radioactivity after the different exposure and postexposure treatment regimens. These are raw data uncorrected for natural decay of ^{51}Cr. Two problems exist with these decay curves. First, since the data in Figure 45 show that external thoracic measurements reflect both lung and GI tract radioactivity until about 30 hours post deposition, the early part of the decay curve in Figure 46 reflects passage of the radioactive particles through the GI tract more than lung clearance. Second, during the approximate interval of 100-300 hours after deposition, the decay appears slower than the decay of the ^{51}Cr itself, which is impossible. This error was introduced by the misalignment of the rat's lungs with the ring shaped detector as the rats grew, requiring movement of the tailgate. One rat which

did not require tailgate movement did not show this slower decay phase. The last 600 hours of the decay curves accurately reflect the decay of the lung's radioactivity because no tailgate adjustments occurred.

TABLE 99. EFFECT OF A SIMULTANEOUS EXPOSURE TO CO_2 AND RADIOLABELED MICROSPHERES ON THE DEPOSITION PATTERN OF THE MICROSPHERES WITHIN THE RAT LUNG: VERTICAL PLANES

	Vertical Plane	Evenness Index; $\bar{X} \pm$ S.E., N = 12			Significant ($p < 0.05$)[a]	
		Air	3% CO_2	5% CO_2	CO_2	Plane
Left Lung	111-131	1.61 ± 0.06	1.21 ± 0.12	1.14 ± 0.06		
	112-132	0.91 ± 0.03	0.98 ± 0.03	0.96 ± 0.03		X
	113-133	0.84 ± 0.03	0.90 ± 0.04	0.90 ± 0.03		
	114-134	1.32 ± 0.06	1.22 ± 0.12	1.46 ± 0.12		
Apical Lobe	211-221	1.49 ± 0.06	1.23 ± 0.08	1.39 ± 0.07		
	212-222	1.31 ± 0.07	1.35 ± 0.08	1.22 ± 0.12		
Cardiac Lobe	311-321	.06 ± 0.06	0.86 ± 0.04	0.92 ± 0.06		
	312-327	0.85 ± 0.05	0.77 ± 0.05	0.86 ± 0.06		X
Diaphragmatic Lobe	411-431	0.93 ± 0.05	1.05 ± 0.10	0.94 ± 0.05		
	412-432	0.98 ± 0.04	0.91 ± 0.03	0.86 ± 0.03		
	413-433	1.00 ± 0.04	0.88 ± 0.03	0.85 ± 0.03		
Azygous Lobe	511-521	0.64 ± 0.07	0.85 ± 0.03	0.74 ± 0.08		
	512-522	0.68 ± 0.05	0.84 ± 0.09	0.60 ± 0.06		

[a] 2-Way ANOVA

TABLE 100. EFFECT OF A SIMULTANEOUS EXPOSURE TO CO_2 AND RADIOLABELED MICROSPHERES ON THE DEPOSITION PATTERN OF THE MICROSPHERES WITHIN THE RAT LUNG: LOBES, RIGHT AND LEFT LUNG, TOTAL LUNG AND TRACHEA

	Evenness Index; $\bar{X} \pm$ S.E., N = 12			Significant ($p < 0.05$)[a]	
	Air	3% CO_2	5% CO_2	CO_2	Lobe/Lung
Left Lung	1.03 ± 0.02	1.08 ± 0.04	1.12 ± 0.03		
Apical Lobe	1.40 ± 0.05	1.29 ± 0.07	1.30 ± 0.07		X
Cardiac Lobe	0.95 ± 0.04	0.82 ± 0.03	0.89 ± 0.04		
Diaphragmatic	0.94 ± 0.03	0.95 ± 0.03	0.88 ± 0.03		
Azygous Lobe	0.65 ± 0.05	0.74 ± 0.06	0.67 ± 0.06		
Right Lung	0.97 ± 0.01	0.95 ± 0.02	0.92 ± 0.02		X
Left Lung	1.03 ± 0.02	1.08 ± 0.04	1.12 ± 0.03		

[a] ANOVA

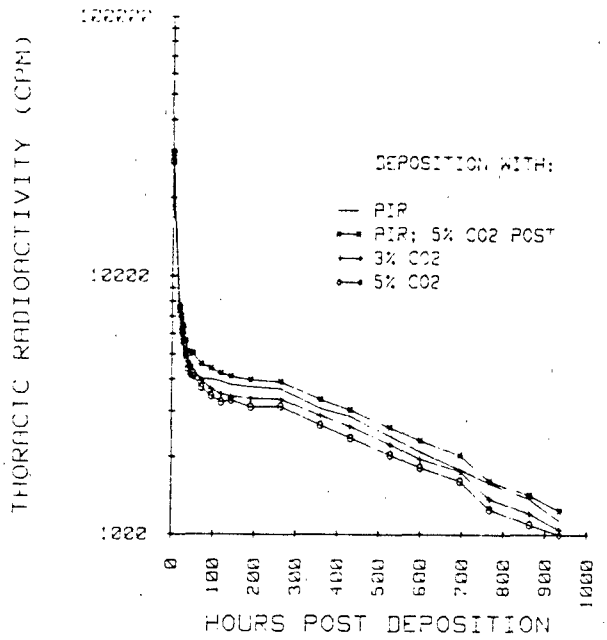


Figure 46. Decay of external thoracic radioactivity with time after simultaneous deposition of radiolabeled microspheres with air, 3 or 5% CO_2 or with air followed immediately with a 5% CO_2 exposure.

Regression analyses for the last 600 hours of the decay curve showed a lower intercept for the CO_2 exposed rats, indicating that fewer particles were deposited in the peripheral lung than in the larger airways. This difference is not due to CO_2 effects on clearance as the 5% CO_2 exposure immediately after the deposition exposure had no effect. Therefore, this was an effect induced during deposition. The half lives for all the treatment groups were not statistically different and had a mean value of 1073 ± 111 hours (X ± S. E., N = 60).

In order to accurately measure lung clearance in the early postexposure period, another experiment was conducted. In this study, the radioactivity was measured at various times during 40 days in the lungs and trachea excised from three rats at 0, 6, 12, 18, 24, and 30 hours postdeposition from both an air and 5% CO_2 exposed group. After 30 hours, the thoracic radioactivity was measured serially in eight rats from each group with the position of

the rat thorax stabilized during growth. Figure 47 shows the decay of radioactivity from the lung through 934 hours postdeposition. An equipment malfunction was the reason no data were collected between 130 and 350 hours. Table 101 shows the half lives for the three components needed to model these decay curves. The CO_2 exposures are again seen to alter the deposition pattern but do not alter the half lives of any component or decrease their variability. Averaging all the data, the three components seen in the lung clearance curve have mean half lives of 18.8 ± 4.7, 102 ± 220 and 1068 ± 123 hours (X ± S. E.).

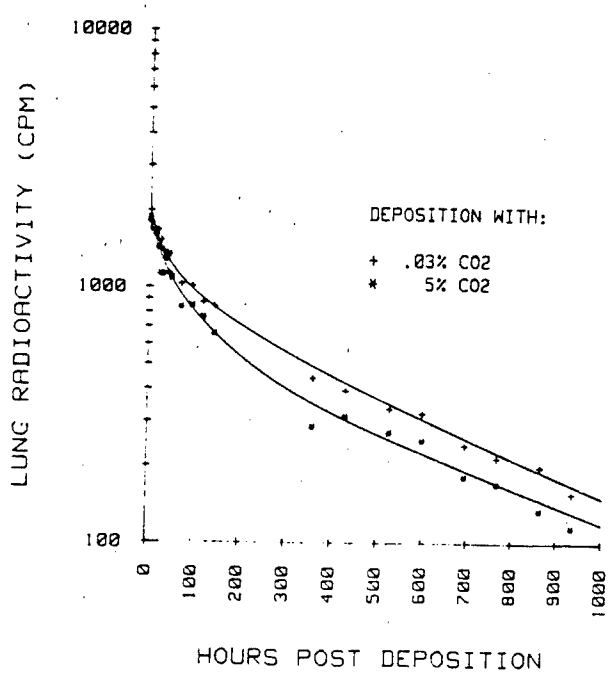


Figure 47. Decay of lung radioactivity with time after deposition of radiolabeled microspheres with air or 5% CO_2.

Discussion

The apex-to-base gradient in particle deposition and the preferential deposition in the apical lobe have been reported previously (Brain et al., 1976; Sneddon and Brain, 1981; Stokinger et al., 1951; Valberg et al., 1982). The elevated inspired CO_2 levels did not statistically alter this gradient. However, if we

consider only the terminal sections of the left lung, which has the most sections and hence best resolution, the CO_2 exposures produced a shift in particle deposition from the apex to the base sections.

TABLE 101. THREE COMPONENT ANALYSES OF LUNG RADIOACTIVITY DECAY AFTER DEPOSITION OF THE RADIOLABELED MICROSPHERES WITH AIR OR 5% CO_2

	Air		5% CO_2	
Component	Intercept, Counts/Min.	Half Life, Days	Intercept, Counts/Min.	Half Life, Days
1	1029 ± 96[a]	2.4 ± 0.8	888 ± 122	2.3 ± 0.3
2	840 ± 49	37.7 ± 4.6	745 ± 89	38.0 ± 2.8

a: $\bar{X}$ ± S.E., N = 8

The clearance of the radiolabeled particles from the lung showed a three-component exponential decay curve. Bianco et al. (1980) found only a single component throughout 300 hours postexposure but they had removed the trachea and large bronchi from the lungs prior to counting and an early component representing clearance from these removed areas would be missed. The first component in our decay curve appears to be due to clearance from the trachea and large airways and the latter two components are from the peripheral lung and smaller airways.

Chan et al. (1981) found a two-component decay curve fit. The first component had a half-life of 24 hours which approximates the first component in the current study. Their second component of 1488 hours is notably longer than we found. However, their procedure used ^{14}C labeled hexadecane which, as a liquid, might have different solubility, metabolism, or binding in the lung such that some of it might not be removed by the clearance mechanisms. Chan's experimental animals were Fischer 344 rats which were found to have a different clearance curve from Long-Evans rats by Ferin and Morehouse (1980). Sprague-Dawley rats were used in our study as were those used by Kenoyer et al. (1981) and Bianco et al. (1980). Therefore, a strain difference may have produced this difference in the late clearance component.

The concept of collection of feces for the first 50 hours was originated by others (Frager et al., 1979; Kenoyer et al., 1981; Phalen et al., 1980) in an attempt to measure the clearance of the

radiolabeled particles during a period of time when the amount of nonpulmonary radioactivity confounded external thoracic measurements of lung radioactivity and decay. The need for an alternative approach to thoracic measurements has been verified in our study. The time for excretion of 50% of the radioactivity excreted during the first 50 hours postdeposition calculated in our study is in agreement with previously published results (Frazer et al., 1979; Kenoyer et al., 1981). However, when this approach was used in previous studies to test for any pollutant effect on clearance, the pollutant exposure started 1 hour after deposition. Results from our study have shown 63% of the particles deposited in or on the rat are already in the GI tract prior to the pollutant exposure. Consequently, the most accurate method for measuring lung clearance for the first 30 hours postdeposition is excision of the trachea and lungs. After 30 hours, serial thoracic measurements accurately reflect clearance of radiolabeled microspheres from the lung.

In summary, clearance of radiolabeled microspheres from the lungs of Sprague-Dawley rats through 934 hours postdeposition can be represented by a three component exponential decay curve with half-lives of 19, 102, and 1068 hours. Elevated inspired CO_2 levels increase ventilation by increasing tidal volume and seem to induce greater deposition in the base section of the lung and in the larger airways but do not alter clearance rates or reduce clearance variability. Excision of the trachea and lungs for the first 30 hours postdeposition followed by serial measurements of thoracic radioactivity using a ring-shaped detector provides a quantitative estimate of clearance of insoluble radiolabeled microspheres from the lungs of rats.

PULMONARY GAS EXCHANGE AND CLEARANCE OF MICROSPHERES AFTER EXPOSURE TO JP-8

As part of the subchronic inhalation toxicity study of JP-8 control rats and those exposed to the 1000 mg/m^3 concentration continuously for 90 days were used to assess pulmonary effects of exposure. Indices of pulmonary mechanics, dynamics, and gas exchange were measured after 1 and 80 days of exposure and again at 42 and 65 days postexposure. Measurements were also made of the ability of the lung to clear radiolabeled microspheres deposited 1 hour after termination of the 90 day exposure.

The radiolabeled microsphere deposition and clearance measurement systems are described separately in this report.

Pulmonary Mechanics, Dynamics and Gas Exchange

Plethysmograph

A whole body constant pressure plethysmograph was constructed measuring 4.5" x 4.5" x 10". Flow rates were determined by measuring the pressure drop across 8 layers of 400 mesh stainless steel cloth, 0.75" in diameter, and volume was obtained by integration. A system of straight through 3/8" diameter computer controlled solenoid valves and bidirectional valves allowed computer controlled switching of the tracheal catheter to: (1) a respirator for animal hyperventilation to produce apnea; (2) positive or negative pressure reservoirs for forced maneuvers; (3) a stopcock for CO diffusion gas mixture incertion and withdrawal, or; (4) a flow-through reservoir containing a 4% Halothane/96% O_2 mixture for anesthesia. The 20 liter positive and negative reservoirs are regulated at +40 and -40 cm H_2O, respectively.

After anesthetization in a Halothane/O_2 chamber, the rat was intubated using a modified 16 gauge intravenous catheter. The rat was then placed within the plethysmograph where it breathed the Halothane-O_2 mixture through a stainless steel tube which connects the tracheal cannula through the plethysmograph wall to the valve system outside (see above). An esophageal catheter made from a shortened .035" O.D. infant feeding tube was then inserted and transpulmonary pressure was measured using a differential pressure transducer with the reference side connected to the airway via a second catheter. Rectal temperature was continuously measured.

Resistance and Dynamic Compliance

After intubation, the tracheal tube was attached through the plethysmograph wall to an open circuit Halothane-O_2 mixture and the Halothane level regulated to stabilize the respiration and concurrently anesthesia level at 55-65 breaths/minute. The resistance and dynamic compliance were then calculated for 10 breaths using transpulmonary pressure and flow integrated tidal volume.

Quasistatic Pressure Volume Curve

After stabilization at 55-65 B/min, to standardize anesthesia level, the rat was hyperventilated to produce apnea and standardize the lung volume history. A slow inspiration (3 ml/sec) was then made to TLC (+25 cm H_2) and then a slow expiration (3 ml/sec) was

made to RV (-25 cm H_2). The transpulmonary pressure and flow integrated lung volume were sampled by the computer and the slope of the P-V curve between 0 and +7 cm H_2 was calculated. The P-V curve was also plotted.

CO Diffusion

After stabilization of the respiration rate and induction of apnea, the lungs were inflated with a gas mixture of 0.5% carbon monoxide, 0.5% neon, and the remainder of nitrogen. The volume injected with a syringe was that volume previously determined as the difference between FRC (lung volume during apnea) and TLC (by definition, +25 cm H_2). After 6 seconds the mixture was withdrawn from the lung and the last 2 ml (alveolar sample) was analyzed by gas chromatography for CO and Ne concentrations. The D_{LCO} was calculated and corrected for space by the computer using standard formulas (Takezawa et al., 1980).

Gas Analysis

Neon and carbon monoxide concentrations were analyzed using gas chromatography. A molecular sieve 13X, 3 mm X 2 meter stainless steel column was used to separate the gases using helium as the carrier gas at a flow rate of 30 ml/min. Oven temperature was 50°C isothermal.

Clinical Hematology

At termination of the experiment, the thoracic cavity was opened and 3 ml of blood was drawn from the inferior vena cava. The blood was mixed with EDTA and analyzed (Coulter Counter, Hialeah, FL).

Experimental Animals

Fischer 344 male rats (Charles River), 175-225 g, were used. Quality control examinations during the quarantine period included bacteriology, gross and histopathology, and showed the animals to be in good health. The animals had food and water ad libitum except during the radiolabeled aerosol exposure and counting periods. Food was Purina Formula #5008, and the water was softened and did not exceed 17 ppm hardness measured as calcium carbonate.

Results

Pulmonary Clearance

The decay of lung radioactivity with time depicting clearance of the deposited radiolabeled microspheres from the lung is shown in Figure 48. Both the control and JP-8 exposed rats showed a three-component clearance curve from 0 through 934 hours after deposition.

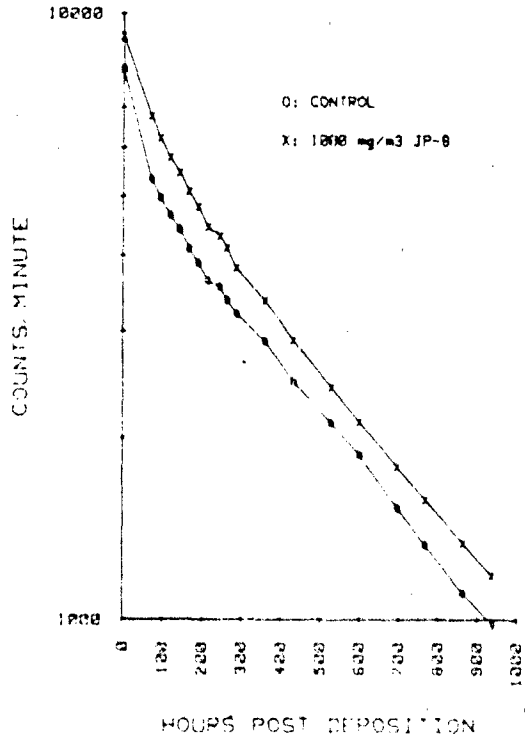


Figure 48. Clearance of radiolabeled particles deposited in the lung after a 90 day continuous exposure to 0 to 1000 mg/m^3 petroleum JP-8.

A previous study included in this annual report has shown that external thoracic counts of lung radioactivity during the first 30 hours postdeposition are confounded by radioactivity in the gastrointestinal tract and on the rat's fur. During that study fecal samples were collected every few hours by placing the rats in holding tubes which induces defecation and consequently accelerates

transport through the gastrointestinal tract. In addition, the detector was collimated to reduce measurements of counts originating in the lower intestinal area. During the present study the rats were not placed in tubes for feces collection, and a larger detector was used which was not collimated to reduce intestinal counts. As a consequence, external thoracic counts did not accurately reflect excised lung counts in the few rats used for this determination until approximately 94 hours postdeposition. Therefore, it was not possible to calculate the clearance half-time in the first component, which was over by the time external thoracic measurements were accurate.

The kinetic constants of the 3 components are given in Table 102. These and the plots in Figure 48 demonstrate that the slopes of control and exposed animal clearance curves are equal throughout the postexposure period and, thus, that the clearance was unaffected by exposure. The deposition, as reflected in the initial count, was higher in exposed animals, and the difference was maintained throughout the measurement period. This constant difference implies that more microspheres were deposited in the deep lung or alveolar regions of exposed animals since an increase in deposition in a region would have been eliminated by early clearance causing the two curves to superimpose.

TABLE 102. EFFECT OF A 90 DAY CONTINUOUS EXPOSURE ON DEPOSITION AND CLEARANCE HALF TIMES OF RADIOLABELED MICROSPHERES IN LUNGS OF RATS

	Component 1		Component 2		Component 3	
	Intercept Counts/min	Halflife HR	Intercept Counts/min	Halflife HR	Intercept Counts/min	Halflife HR
Control,	1037	N/A	2021 ± 370[a]	40 ± 5	5531 ± 372	853 ± 24
Exposed,	338	N/A	2680 ± 482	50 ± 4	6612 ± 265[b]	845 ± 54

[a] $\bar{x}$ ± SEM
[b] Significantly different from control, $p \leq .01$

Pulmonary Function

The pulmonary function test results are presented in Table 103. A 24 hour exposure to 1000 mg/m^3 JP-8 produced no significant

alterations in the measured parameters. After 80 days of continuous exposure, the same rats were tested and pulmonary test parameters were the same as controls except for a decrease in weight, hemoglobin (HGB), and carbon monoxide (CO) diffusion capacity (D_L/V_A) which was a reflection of the slight decrease in hemoglobin in the exposed rats. Test values at 42 days postexposure in these same rats again showed the slight anemia and decrease in CO diffusion. However, at this time other test values showed a pattern of restrictive lung disease with decreases in lung volumes and compliance. Because the animals were killed at this time for lung weight measurements and other studies, further testing of these same rats was impossible. In order to confirm this pattern of restrictive lung disease, rats scheduled for standard pathologic evaluation at 65 days postexposure were also tested. These results, also presented in Tables 103a and 103b, showed the pattern of restrictive lung disease but the level of significance of the test values was below .05. It is not possible to judge the meaning and importance of the pulmonary function results. Tests will be repeated during and after a planned exposure to Shale JP-4 to determine whether it will lead to pulmonary function decrements.

TABLE 103a. EFFECT OF A 90 DAY CONTINUOUS EXPOSURE TO 1000 mg/m^3 JP-8 ON PULMONARY FUNCTION AND BLOOD PARAMETERS OF MALE FISCHER 344 RATS

	24 Hour Exposure		80 Day Exposure	
	Control	Exposed	Control	Exposed
VT, ml[a]	0.97 ± 0.03	1.02 ± 0.06	1.45 ± 0.09	1.52 ± 0.12
R dyn, cm H_2O/ml/sec[a]	0.17 ± 0.02	0.16 ± 0.01	0.20 ± 0.02	0.20 ± 0.02
C dyn, ml/cm H_2O[a]	0.35 ± 0.02	0.36 ± 0.03	0.47 ± 0.04	0.50 ± 0.05
C static, ml/cm H_2O	0.56 ± 0.01	0.59 ± 0.02	0.62 ± 0.04	0.57 ± 0.15
DL, ml/min/cm H_2O	0.19 ± 0.01	0.21 ± 0.01	0.22 ± 0.01	0.19 ± 0.01
DL/VA, ml/min/cm H_2O/ml	0.023 ± 0.001	0.024 ± 0.001	0.024 ± 0.001	0.022 ± 0.001[b]
TLC, ml				
VC, ml				
IRV, ml				
ERV, ml				
FRC, ml[d]				
RV, ml				
IC, ml				
Hgb, g/dl			15.6 ± 0.3	14.7 ± 0.2[b]
Hct, %			43.6 ± 0.8	38.3 ± 0.5[c]
MCHC, g/dl			35.8 ± 0.2	38.3 ± 0.3
WBC x 10^3			5.8 ± 0.3	7.0 ± 0.3[b]
RBC x 10^6			8.5 ± 0.1	7.5 ± 0.1[c]
Weight, g	191 ± 2	187 ± 3	327 ± 2	308 ± 5[c]
Wet/Dry Lung Weight				
N	14	14	14	14

[a]Determined at f = 80 ± 5 B/M
[b]Significant, $p \leq .05$
[c]Significant, $p \leq .01$
[d]Apneic lung volume after hyperventilation

TABLE 103b. EFFECT OF A 90 DAY CONTINUOUS EXPOSURE TO 1000 mg/m^3 JP-8 ON PULMONARY FUNCTION AND BLOOD PARAMETERS OF MALE FISCHER 344 RATS

	42 Days Post		65 Days Post	
	Control	Exposed	Control	Exposed
VT, ml[a]	1.66 ± 0.05	1.42 ± 0.07[c]	1.50 ± 0.07	1.51 ± 0.10
R dyn, cm H_2O/ml/sec[a]	0.24 ± 0.03	0.24 ± 0.02	0.22 ± 0.01	0.23 ± 0.02
C dyn, ml/cm H_2O[a]	0.50 ± 0.03	0.40 ± 0.03[c]	0.45 ± 0.03	0.53 ± 0.07
C static, ml/cm H_2O	1.01 ± 0.07	0.93 ± 0.07	0.94 ± 0.05	0.69 ± 0.07[c]
DL, ml/min/cm H_2O	0.25 ± 0.01	0.21 ± 0.02[b]	0.29 ± 0.02	0.28 ± 0.01
Dl/VA, ml/min/cm H_2O/ml	0.022 ± 0.001	0.021 ± 0.001	0.021 ± 0.001	0.024 ± 0.001
TLC,ml	15.2 ± 0.02	13.7 ± 0.7[b]	15.9 ± 0.4	15.1 ± 0.4
VC,ml	12.9 ± 0.2	11.2 ± 0.6[c]	13.5 ± 0.6	12.6 ± 0.5
IRV, ml	9.8 ± 0.2	8.5 ± 0.5[b]	9.9 ± 0.5	9.2 ± 0.3
ERV, ml	1.4 ± 0.2	1.2 ± 0.3	2.1 ± 0.3	1.9 ± 0.4
FRC, ml[d]	3.8 ± 0.1	3.7 ± 0.2	4.5 ± 0.3	4.4 ± 0.2
RV, ml	2.4 ± 0.2	2.5 ± 0.3	2.5 ± 0.5	2.5 ± 0.5
IC, ml	11.5 ± 0.2	10.0 ± 0.5[b]	11.4 ± 0.5	10.7 ± 0.3
Hgb, g/dl	15.0 ± 0.1	14.3 ± 0.1[c]	14.8 ± 0.2	13.6 ± 0.3[c]
Hct, %	39.8 ± 0.4	38.0 ± 0.3[c]	38.9 ± 0.5	35.7 ± 0.7[c]
MCHC, g/dl	37.7 ± 0.3	37.6 ± 0.1	37.9 ± 0.5	38.1 ± 0.4
WBC x 10^3	5.4 ± 0.2	5.4 ± 0.2	4.7 ± 0.2	4.7 ± 0.3
RBC x 10^6	7.9 ± 0.1	7.5 ± 0.1[c]	7.7 ± 0.1	7.0 ± 0.1[c]
Weight g	334 ± 4	311 ± 6[c]	337 ± 5	327 ± 5
Wet/Dry Lung Weight	5.35 ± 0.10	5.44 ± 0.06		
N	14	14	10	10

[a]Determined at f = 60 ± 5 B/M
[b]Significant, $p \leq .05$
[c]Significant, $p \leq .01$
[d]Apneic lung volume after hyperventilation

CHAMBER CONTAMINANT ANALYSIS AND CONTROL SYSTEM

Previously, the sampling rates and alarms for high and low concentrations within the Thomas Dome Exposure Chambers had been designed and constructed for each individual chamber. Mechanical timers controlled sampling frequency, and mechanical high and low alarm points were set on the strip chart recorder, to be activated when the recorder pen reached these points. Construction and adjustment of these systems was a tedious, time consuming operation, and the possibility existed that they might drift from the set points. In order to overcome these deficiencies, a microprocessor based system was designed and constructed for control and monitoring of the chamber contaminant analysis system. Some of the features

which the system includes are individually-set timers for each chamber, adjustable high and low alarm set-points, adjustable sample-on delay times, and display of all parameters on a video screen. As shown in Figure 49, the system consists of a central controller, video display terminal, a voltage input circuit card, 4 voltage output control cards, 8 locally-mounted control and monitoring boxes, and appropriate sample control valves or solenoids.

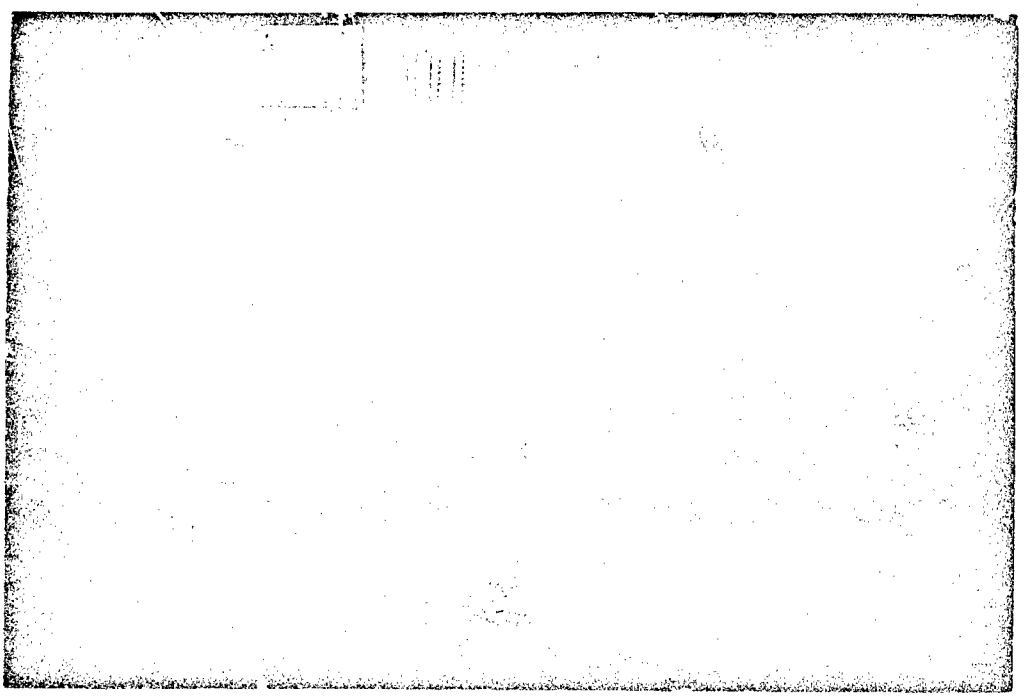


Figure 49. Chamber contaminant analysis and control system.

The central controller is a microprocessor-based card system manufactured by the Transwave Corporation. The controller card contains a circuit card powered by a National 8073 microprocessor. Application programs written in the Basic language may be stored in the controller memory and used to operate the system. An applications program of this type was written to provide control of the chamber timer and alarm system.

The central controller card, containing the microprocessor chip, is connected to a 16-channel analog-to-digital voltage converter card and four 8-channel relay voltage output cards by way of a coaxial cable-serial data output line. This 2-way data communications line sends and receives data from each of the peripheral input-output cards. Eight of the 16 analog-to-digital channels are connected to the analytical system recorders, leaving the remaining eight channels for future applications. Normally these input signals are calibrated to equal full-scale deflection of the analytical system recorders which are proportional to contaminant concentrations. The signal may then be displayed on the video display terminal for observation. This signal is also electronically compared to pre-set values for each channel and will activate visual and audible alarms if contaminant levels exceed a fall below specified limits.

The central controller card is connected through the serial data line to each of four 8-channel relay output cards. Each card supplies control outputs for 2 chambers. The four control outputs supplied to each chamber are:

1. Contaminant Sample Valve
2. Alarm Delay
3. High Alarm
4. Low Alarm

A separate RS-232-type serial data line from the central controller is connected to a video display terminal. The applications program is designed to provide a display as shown in Figure 50. The columns displayed are:

1. Dome Number
2. Sample Period
3. Alarm delay
4. High Set Point
5. Low Set Point
6. Chart Reading
7. Timer Reading
8. Timer Period

The video-display terminal contains a standard keyboard which may be used to input information for controlling the system. The program will query the operator with English language questions for the sample period in minutes, the sample delay in minutes, the high

and low set points in chart divisions. These values may be entered individually for each chamber. After initiation of the program, the system automatically updates all timers and chart readings for each chamber system. This update is displayed on the video display terminal approximately three times per minute.

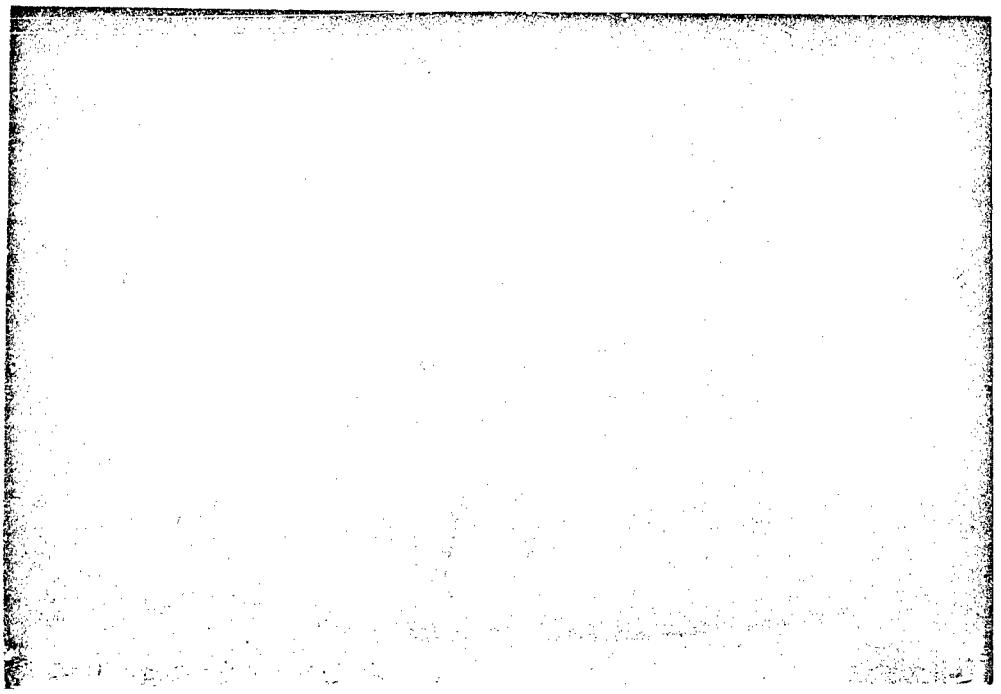


Figure 50. Display terminal readout.

Concurrently with the video display updating, control outputs are sent to the locally-mounted control and monitoring boxes. These boxes as shown in Figure 51, contain lamps to indicate sample on, baseline, alarm off, high alarm and low alarm conditions. A switch is also mounted on the front panel to provide for manual or automatic control of the sample control valve. A push-button switch is included to silence the audible alarm which is activated whenever there is a low or high alarm. Mounted on the rear of the enclosure are outlets for connecting an electronically operated actuator valve, 110VAC solenoid outlets and the connecting cable from the central computer controller.

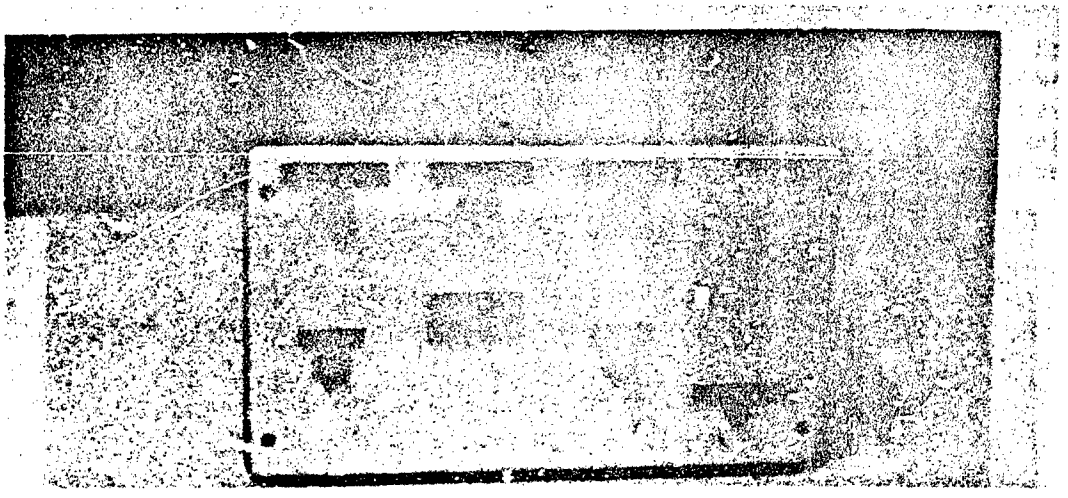


Figure 51. Alarm timer control boxes.

There are several modifications planned for the system to provide for hard-copy printer output of the video display information and also to provide for storage and hard-copy printout of the concentration data received from each chamber recorder.

CHAMBER TECHNICIAN TRAINING PROGRAM

The Thomas Dome Standard Operating Procedures Training Program for new technicians and the routine Monthly Emergency Training Procedures have been revised since the last annual report. The subjects covered in the training program for new technicians are as follows:

I. Orientation
   - A. Laboratory mission
   - B. Job responsibilities
     1. Introduction to SOP's
     2. Introduction to lab operations
   - C. Personnel responsibilities
     1. Good Laboratory Practice procedures

II. Standard Operation of Chambers
   A. Observer A normal routine
      1. Dome start-up
      2. Establish flow
      3. Normal readings
      4. Dome entry operation
   B. Observer B checklist
   C. Dome entrant duties
      1. Dome entry operation
      2. Dome cleaning and cage changes
   D. Dome cap raising and lowering

III. Mechnical Equipment
   A. Vacuum pump failure
      1. Facility A pump
      2. Facility B pump
      3. Observer duties
   B. Air compressor failure
      1. Main air compressor
      2. Back-up air compressors
      3. Air dryers
      4. Observer duties
   C. Complete power failure
      1. Facility A procedures
      2. Facility B procedures
      3. Observer duties

   D. Air supply fan failure
      1. Main supply fan
      2. Back-up supply fan
      3. Observer duties
   E. Waste catch tank draining
      1. Transfer dome to tank
      2. Emptying of tank
   F. Toxsys system data collection SOP

IV. Emergencies
   A. Fire in dome during entry
      1. Observer A duties and options
      2. Observer B duties and options
      3. Dome entrant duties
   B. Fire in dome - no entrant
      1. Observer A duties and options
      2. Observer B duties and options

C. Fire in airlock during entry
   1. Observer A duties and options
   2. Observer B duties and options
   3. Dome entrant duties
D. Fire in exposure laboratory
   1. Observer A duties and options
   2. Observer B duties and options
E. Rescue of incapacitated dome entrant
   1. Rescue criteria
   2. Observer A duties
   3. Observer B duties
F. Operation of Scott Air Pak (SCBA)
   1. Criteria for use
   2. Procedures
G. Miscellaneous emergencies
   1. Criteria requiring immediate action
H. Building 429 alarm
   1. Observer A duties

The monthly Emergency Training Procedures Program has also been revised. Training in the procedures is conducted by the Senior Technicians on each shift. Periodic written examinations are given by the Principal Technician to all Chamber Technicians. Revisions of any procedure and/or retraining is made by the Principal Technician as the need arises. Listed below is the schedule for the training procedures and examinations given during the past year. Documentation of all practical, oral, and written examinations is also maintained.

Date		Procedure
*January	-	Vacuum Pump Failure
February	-	GLP Procedures and Toxicology SOP's
*March	-	Air Compressor Failure
April	-	Fire in Exposure Laboratory During Entry
*May	-	Operation of Scott Air Pak
June	-	Fire in Dome During Entry
*July	-	Supply Air Fan Failure
August	-	Building 429 Alarm
*September	-	Rescue of Incapacitated Dome Entrant
October	-	Complete Power Failure
*November	-	Fire in Airlock During Entry
December	-	Air Compressor Failure

* Written Examinations

The number of Chamber Technicians AALAS certified at each level is:

Laboratory Animal Technologists	3
Laboratory Animal Technicians	4
Assistant Laboratory Animal Technicians	3

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The research program of the Toxic Hazards Research Unit (THRU) for the period of June 1982 through May 1983 is reviewed in this report. Chronic toxicity and oncogenic studies were carried out with hydrazine, Otto Fuel II, JP-7, JP-8, and JP-TS. A series of acute toxicity studies was conducted on a variety of chemicals and chemical agents used by the Army, Air Force, and Navy. Neurotoxicity and subchronic inhalation studies were conducted on several hydraulic fluids.

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Dermal
Shale Oil Fuels
Petroleum Fuels
Otto Fuel II
Neurotoxicity
Intraperitoneal
Antimony Thioantimonate
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